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PHYTOPATHOLOGY

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NOVEMBER, 1935

RUST RESISTANCE IN ANTIRRHINUM[1]

E. B. MAINS

(Accepted for publication January 21, 1935)

Snapdragon rust, *Puccinia Antirrhini,* was described by Dietel and Holway (6) from specimens of rusted *Antirrhinum majus* collected by W. C. Blasdale at Berkeley, California, in 1895. Blasdale (3) in 1903 stated that it was common in the region around San Francisco Bay but that he had no knowledge of its occurrence elsewhere in the State. At that time the rust appeared every year in his garden with such severity that the infected plants of snapdragon were killed shortly after reaching the flowering stage. Peltier (15) reported the rust as severe in the vicinity of Chicago in 1913. By 1921 (7, 15), it was widely distributed throughout temperate North America. It now is known (2) in 24 States of the United States, 5 Provinces of Canada, and in Bermuda. Until recently (1933) it has been unknown in Europe. It is now reported (9) in England, Belgium and Germany.

Blasdale (4) found that, in addition to *Antirrhinum majus,* the rust occurred on *A. Nuttallianum* and *A. virga* in California. He reports success in infecting *A. vagans, A. assurgens, A. nudicum, Linaria amethystina, L. delphinifolia, L. bipartita, L. melanthera,* and *L. macrocanna.* Arthur (2) reports the rust on *A. Nuttallianum* and *A. virga* in California. He also includes rust on *Cordylanthus* (*Adenostegia*) *filifolius, C. pilosus* and *C. rigidus.* Because *Antirrhinum majus* is native of the Mediterranean region, where the rust is unknown, and became rusted only after snapdragons were introduced into California, it is evident, as Blasdale (3) has suggested, that the rust was indigenous on Californian species of Antirrhinum and found snapdragons a congenial host where the latter were introduced.

Studies concerning the host range of the rust have been reported also by Peltier (15) and Emsweller and Jones (8). Peltier states that he inoculated several species of Antirrhinum (not specified), a large number of commercial varieties of *A. majus, Linaria vulgaris* and a number of species and commercial varieties of Linaria (not specified). All the varieties of *A.*

[1] Papers from the Department of Botany and Herbarium of the University of Michigan No. 520. These investigations were started while the writer was a member of the Department of Botany, Purdue Agricultural Experiment Station, and have been continued at the University of Michigan.

majus were severely infected. A slight infection was obtained on *A. maurandioides* Gray. "No infection was obtained on *L. vulgaris* or on any other species or commercial varieties of Linaria. This was true also of Antirrhinum species with the exception of *A. majus*. . . ." Emsweller and Jones studied a number of species of Antirrhinum collected by E. Baur in Spain. A collection of *A. Ibanjezii* and a collection of *A. siculum* were resistant. A collection of *A. tortuosum* was susceptible. A strain of *A. glutinosum* and of *A. molle* proved to be resistant, while a second strain of each species was susceptible. A collection of *A. hispanicum* contained both resistant and susceptible plants.

Puccinia Antirrhini is apparently heteroecious. Mains (12) and Hockey (10) have germinated the teliospores. Snapdragons were inoculated without obtaining infection. The hosts for the aecial stage are unknown.

In the United States the rust has become an important factor in the culture of snapdragons. For a time it was specially serious in greenhouses where snapdragons were propagated by cuttings. Peltier (15) showed that the rust is not seed-carried. With the abandonment of propagation by cuttings and the adoption of such practises as subirrigation, regulation of temperature, etc. (7, 15), the rust is less serious in the greenhouse.

It still remains more or less important in the garden. When it does not result in the death of infected plants, it renders them unsightly, naturally a very undesirable condition for an ornamental. Also, in California, the rust often has important economic results, since most of the seed used in North America is grown there. Thus, in 1921, D. G. Milbrath wrote that the damage was the most that he had ever observed. One large seed grower lost his entire crop, valued at $10,000, and in other fields the crop was only 25 per cent of the normal.

Sulphur compounds, specially sulphur dusts, have been used successfully to greatly reduce the development of the rust (5, 7, 13). Butler (5) found that successful control varied from year to year, apparently correlated with temperature. Rotation of crops, early planting, withholding of water, have been employed in the seed-production districts of California (8). Although reducing the damage to some extent, none of these methods has been entirely satisfactory.

It is obvious that highly resistant strains would be the most desirable solution of the difficulty. Peltier (15) tested a large number of commercial varieties, all proving to be susceptible. Doran (7) reports a study of 46 varieties. His results are difficult to interpret. Under the heading "Scale, relative numbers" he has assigned values, 0 to 100, to the various varieties. He states that, while the most resistant varieties were not absolutely resis-

tant, they were the nearest to it among snapdragons. It would appear, therefore, that the designation "0" was not used to indicate immunity.

Little or no evidence of resistance has been noted by other investigators. D. G. Milbrath has informed the writer that in 1926 he inspected a large number of fields of snapdragons in California without finding any individuals outstanding for resistance. Emsweller and Jones (8) state that in 1929 a search for resistant individuals in the fields of the large seed growers of California was unsuccessful, and Emsweller, in a letter to the writer, has stated that over a period of 15 years no degree of resistance had been noted in the California seed fields.

The writer's studies started in 1922 with 2 selections that showed some resistance. The progress attained in developing resistant strains was reported (14) at the Nashville meeting of The American Phytopathological Society, in December, 1927. As a result, requests for seed were received and in 1928 and succeeding years several lines were sent to various investigators. Selections from these have been used by Emsweller and Jones (8) and by White (16) for developing rust-resistant lines of commercial types. The following account summarizes the development of these strains and the results obtained in additional studies.

METHODS

The technic of inoculation has been described in detail elsewhere (11). In brief the method used in greenhouse studies was as follows. Abundant inoculum was maintained on susceptible plants. Such heavily rusted individuals were shaken over the plants to be inoculated, thus dusting the latter with urediospores. They were then atomized with water and covered with muslin which was wetted. If this be done in the afternoon the lower temperatures during the night will result in a high humidity and condensed water on the leaves. In addition to production of conditions of humidity conducive to germination, the lower night temperatures also favored germination. The results obtained from a study of the effect of temperature upon germination of the urediospores and upon infection are not entirely in accord with those reported by Doran (7). The average germinations obtained at various temperatures were as follows: trace at 0° C.; 74% at 7° C.; 94% at 10° C.; 77% at 15° C.; 48% at 19° C.; 25% at 24° C.; 9% at 26° C.; trace at 30° C. These results are supported by infections obtained by inoculating at various temperatures (Fig. 1). Doran (7) found that 10° C. was the optimum temperature for germination, but reports that a decrease or increase of 2° C. resulted in a decrease of 50% in germination.[2] Inoculated

[2] It should be noted that Doran gives a value of 100 for the germination at 10° C. (actual percentage not stated) and records relative values for the results obtained at other temperatures. The discrepancy may possibly be explained by a difference in germinability of the spores employed in the two studies.

FIG. 1. Leaves selected to illustrate infection of susceptible snapdragons by *Puccinia Antirrhini* when inoculated at temperatures (left to right) of 10°, 15°, 19°, 22°, and 30° C.

plants were uncovered after 24–48 hours and infection appeared in 10 to 15 days, depending upon conditions of light and temperature.

In the field heavily infected plants were distributed at intervals throughout the plot. Infection spread from such sources. Susceptible strains were placed at intervals among the resistant selections and served for comparison.

In selecting for resistance, selected individuals were self-pollinated. Most commercial strains of snapdragons are self-fertile. If they are bagged to prevent pollination by insects, usually there is an abundant production of seed. However, difficulty was experienced under field conditions during the summer months when apparently high temperatures in the bags destroyed viability of pollen. As the study progressed it was found desirable to insure the application of pollen to the stigma. For this purpose wooden toothpicks with a broad flat end were found very useful. They can be obtained at little cost. Pollination is accomplished by drawing the pollen from the lower anthers over the stigma. After all the flowers of the spike are pollinated the toothpick is discarded.

RESULTS

This study originated from observations made in the writer's own garden. It was continued for a number of years in the Department of Botany of the Purdue University Agricultural Experiment Station, Lafayette, Indiana, as one of a number of miscellaneous investigations secondary to major projects. Consequently, the progress of the study was somewhat retarded because of limited time and facilities. During the first few years a study was made of varieties and selections of the snapdragon, *Antirrhinum majus*. Later, attention was turned to other species of Antirrhinum and to

the possibility of developing resistant snapdragons from crosses with such species.

COMMERCIAL STRAINS AND VARIETIES OF SNAPDRAGONS

In 1922 snapdragons of mixed colors in the writer's garden became heavily rusted. By autumn most of the plants were killed. However, two

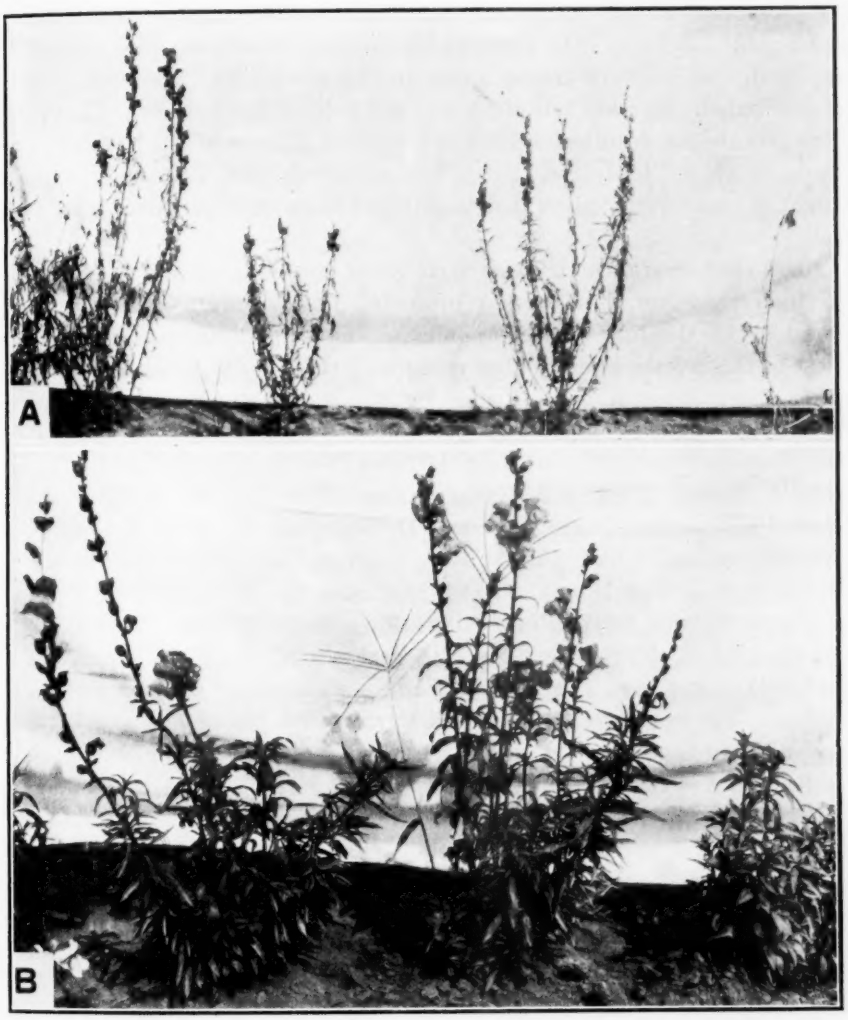

FIG. 2. A. Condition, due to rust, of plants of the variety Maralda in a field planting in September, 1927. B. Condition of resistant selections of the 7–13 strain of snapdragons in September, 1927, in a field planting where commercial strains were severely rusted.

plants survived, although moderately rusted. They were transplanted to the experiment station greenhouse and the flower spikes were bagged and self-pollinated. Young plants raised from seed thus produced were transplanted to the field the next summer and exposed to rust. Considerable variation occurred in the progenies. A number were killed by the rust. The majority were alive at the end of the season, although moderately rusted. A few were only 25 per cent rusted and the uredia were small. Among these were 2 plants, 2–1 and 7–13, from which the most promising lines were later developed. These were transplanted to the greenhouse where they were self-pollinated. Further selections and self-pollination, both in the field and in the greenhouse, resulted in 1928 in a number of lines of the 2–1 and 7–13 strains in which highly resistant plants occurred (Fig. 2). Only a slight amount of rust developed on such plants and the uredia produced were very small.

Each year several commercial varieties of snapdragons were included in the study. Among these were represented most of the colors and types offered by the leading seed companies of the United States. During the course of the investigation the following varieties have been studied: Amber Queen, Apple Blossom, Black Prince, Bronze Scarlet, the Bride, Buff Queen, Butterfly, Buxton, Canary Bird, Captivation, Cerise King, Chamois, Copper King, Cottage Maid, Crimson King, Delicata, Delila, Fascination, the Fawn, Feltham Beauty, Firebrand, Garnet, Giant White, Gloria, Golden Fairy, Golden Pink, Golden Queen, Gotelind, Hybrid Pink, Keystone, Los Angeles, Lovely, Maralda, Mont Blanc, Nelrose, Peerless Pink, Phelps White, Philadelphia Pink, Prima Donna, Purity, Queen of the North, Queen Victoria, Rose Dore, Scarlet, Silver Pink, Snowflake, and Wallflower. Most of these were susceptible, showing few or no differences. All the plants were eventually killed by the rust. In a few varieties an occasional plant was less susceptible. The rust developed more slowly on such plants and they usually survived until the end of the season. Such plants were selected in the varieties Butterfly, Canary Bird, Giant White, Gloria, Golden Queen, Peerless Pink, and Prima Donna. From most of these, after several years of inbreeding, lines somewhat less susceptible than the parent varieties were isolated. These, however, did not approach the 2–1 and 7–13 lines for resistance and, therefore, were not continued. However, one selection, G W D 1, from the variety Giant White, after several years of inbreeding, produced plants equalling those obtained in the 2–1 and 7–13 lines and produced a much better plant and flower type than those of the latter.

Table 1 gives the results obtained in the 1929–1930 greenhouse study of the most promising rust-resistant selections. Although no line had at that time been produced that was uniformly highly resistant, a number showed a marked improvement over commercial varieties as far as rust resistance

TABLE 1.—*Rust reaction in greenhouse of outstanding selections of snapdragons in 1929*

Selections or variety	Number of plants reacting[b]				
	0–t	1	2	3	4
2–1–1–1–G1–F1–G1		45	15		
2–1–1–1–G3–3	1	32		5	
2–1–1–2–G3–4	2	40	16		
2–1–1–3–G2–1	1	42	20	3	
7–13–8–1–G1–F1–G1		34	6	4	
7–13–8–1–G1–F2–3	3	33	8	2	
7–13–8–1–G6–F1–2		15	21	2	
7–13–8–1–G6–F2–F3		6	15	11	11
7–13–19–3–G3–3		48	15		
G W D1–G2–F1–1	1	15	4		
G W D1–G2–F3–3	1	25	5		
G W D1–G3–F1–2	5	35	3		
G W D1–B4–F2–3	8	38			
Nelrose[a]					55
Golden Fairy[a]					45

[a] Varieties from commercial seed included for comparison.

[b] 0–t, no uredia or few very small uredia; 1, few small uredia; 2, moderately rusted, uredia moderate size; 3, fairly heavily rusted, uredia moderate size; 4, severely rusted uredia large.

was concerned. As the inbreeding progressed, more or less sterility developed, especially in the 2–1 and 7–13 lines, which greatly retarded the inbreeding and selection of homozygous, highly resistant lines. This was especially unfortunate, since the number of individuals that could be studied was limited. Also, as a result of these restrictions and the necessity of concentrating efforts on the selection of desired types of resistance, it was not possible to give much consideration to other qualities. The most promising rust-resistant selections, especially in the 2–1 and 7–13 lines, unfortunately proved to be undesirable for commercial purposes. The G W D lines, although not all that could be desired, were the most promising both for rust resistance and other qualities. They produced moderately tall plants with white flowers of good size, fairly well-spaced on the spike.

Consequent upon publication of results reported in 1927 (14), requests for seed of resistant lines were received. It was evident that the utilization of the resistant selections would probably necessitate an extensive study of hybrids made with desirable types, a phase for which time and facilities were not available. It was decided, therefore, to distribute the most promising strains in order that they might be tested in other parts of the country and utilized, by those who so desired, to develop resistant strains of commercial

value. Subsequent efforts in the investigations reported here have been directed mostly in a continuation of the attempt to isolate highly resistant lines from as many sources as possible.

Seed of the most promising lines for resistance have consequently been sent from time to time to investigators in Arkansas, Bermuda, California, Massachusetts, Michigan, and Virginia. The reports received have been as follows: Seven strains were sent to D. G. Milbrath in 1927 and were tested by him at Sacramento, California. He reported that all of these rusted more slowly than the commercial strains with which they were compared. By the end of the season, 4 (G W D1–G2, G W D1–G3, 2–1–1–3–G2, 7–13–19–3–G3) had developed very little rust, averaging less than 30 sori per plant, 2 (7–13–F6–G1, 348–F2–F1) rusted to a greater extent but survived, and only one (2–1–1–3–G1) was seriously affected.

A number of lines were sent to J. B. Kendrick in 1928 and 1929 and were studied by him and by S. L. Emsweller at Colma, and Guadelupe, California. Their notes may be summarized as follows: At Colma the resistant strains were scattered through a commercial planting. All the strains selected for rust resistance were more resistant than the commercial types. Two were outstanding. Although in close association with commercial types that were dying from rust, most of the plants of 2–1–1–1–G3 were still in growing condition and 2 plants had but 2 per cent of the leaves rusted and these bore only 1–3 uredia. Strain 2–1–1–2–G3 was somewhat less resistant. The uredia were fewer and smaller than those on commercial strains. The other 5 strains were more severely infected, although less so than commercial strains.

At Guadelupe, in 1929, the rust was specially prevalent and the strains were subjected to a very severe test. In August only 8 out of 100 plants were dead in strain G W D1–G2–F1. The remainder averaged only 1 uredium per leaf. Only 10 out of 100 plants of G W D1–G2–F3 were dead and the remainder averaged only 2–5 uredia per leaf. Strain 7–13–19–3–G3 had 5 out of 100 plants dead, the remainder averaging 3–4 uredia per leaf. Strain 7–13–F6–G1–1 was similar. Of the other 6 strains, 4 had from 14 to 50 plants killed out of 100 and 2 had all the plants killed by the rust.

In 1930 additional strains were tested at Guadelupe. Too few plants were grown to justify a comparison between strains. However, 11 plants were noted that showed no rust.

The resistant plants in these tests did not meet the standards of the growers in regard to flower and plant types. However, Emsweller and Jones (8), starting with some of the most resistant survivals[3] and crossing

[3] In a letter Professor Emsweller informs me that their fully resistant strains originated from 2–1–1–1–G3 and 2–1–1–2–G3.

with commercial strains, have been successful in developing desirable commercial types possessing marked resistance.

In 1930 a number of strains were tested by Harold E. White at Chatham, Massachusetts. In August, when notes were taken, a number of the commercial varieties with which they were being compared had been completely killed by the rust. Thirty-seven out of 87 plants of the resistant strains showed little or no rust. The most outstanding strains were 2–1–1–1–G1–F1–G1 and 7–13–8–1–G6–F2–F1–G1 and selections from a cross between G W D1 and resistant selections of *Antirrhinum glutinosum,* which will be discussed later.

SPECIES OF ANTIRRHINUM AND RELATED GENERA

Another approach has been through wild species and strains of Antirrhinum. This phase of the investigation was started in 1926. At that time 8 species of Antirrhinum, 1 of Adenostegia and 6 of Linaria were studied. Additional species have been added from time to time as opportunity offered. Seed of a number of species was kindly supplied by E. Baur, F. Brieger, L. A. Waitsinger, and various Botanical Gardens. All the species of Linaria studied, *L. canadensis* Dum.-Cours, *L. Cymbalaria* Mill., *L. dalmatica* Mill., *L. pallida* Tenore, *L. purpurea* Mill., and *L. vulgaris* Mill., gave no evidence of infection. *Adenostegia pilosa* Greene (*Cordylanthus pilosus* A. Gray) also, was found to be highly resistant. It is to be noted that this is given as a host for *Puccinia Antirrhini* by Arthur (2). The results obtained with species of Antirrhinum are given in table 2.

In some instances seed germinated poorly, so that only a few plants were available for study. In such cases the results do not necessarily entirely represent the situation in the strain or the species. In these studies the species *A. asarina, A. Ibanjezii, A. maurandioides,* and *A. virga* were uniformly highly resistant and *A. Barrelieri, A. Coulterianum, A. glandulosum,* and *A. vexillo-calyculatum* were uniformly susceptible. The strains of *A. latifolium* and *A. Valentinum* studied contained a few individuals showing some resistance. *Antirrhinum Orontium,* for the most part, was moderately resistant. *Antirrhinum glutinosum, A. hispanicum, A. molle, A. Nuttallianum, A. sempervirens, A. siculum,* and *A. tortuosum* showed marked differences between or within strains of the species. Thus, in *A. glutinosum,* strain 200 contained highly resistant, moderately resistant, moderately susceptible, and very susceptible individuals, and strains B3 and B8 were uniformly highly resistant. The strains received from E. Baur as wild forms of *Antirrhinum majus* proved very interesting, since strain B6 was highly resistant and strains B7 and B8 contained both highly resistant and susceptible individuals. Emsweller and Jones (8) report resistance in *Antirrhinum glutinosum, A. hispanicum, A. Ibanjezii, A. molle,* and *A. siculum.*

TABLE 2.—*Reaction of species of Antirrhinum to rust*

Species	Accession No.	Number of plants reacting[a]				
		0–t	1	2	3	4
Antirrhinum asarina L.	239	19				
" "	1636	23				
" Barrelieri Bor.	1866					26
" Coulterianum Benth.	611				1	3
" glandulosum Lindl.	603				3	2
" "	612					28
" glutinosum Boiss. & Reut.	200	2	2	1	4	2
" " Lagerfels[b]	B3	8				
" " Orgiva[b]	B4	12				
" hispanicum Chav.	B10					11
" "	1868			1	3	8
" "	1638	2	1		14	3
" "	1634			1	1	13
" Ibanjezii Cartagena[b]	B5	12				
" latifolium Mill.	1128				2	3
" "	1869			1		21
" " Mentone[b]	B12			2	13	
" majus L. Lucena[b]	B6	16				
" " Loja[b]	B7	1				6
" " Cintra[b]	B8	6		15	10	12
" " Zaragoza[b]	B9	12	1	8		14
" maurandioides A. Gray	1121	16				
" molle L. Braganza[b]	B2				5	4
" " Monsech[b]	B1	12				
" Nuttallianum Benth.	605	11		16	6	7
" Orontium L.	1123	4	2	1		
" "	1872			33		
" sempervirens Lapeyr.	1120	8				
" "	1873			1	2	8
" sempervirens[c]	B13	18	26	6		13
" siculum Mill.[b]	B14	3	6	12	17	
" "	Br6				27	
" "	Br7				12	
" "		6	10		1	
" "	1635			27	11	
" "	1874			2		27
" tortuosum Bosc.[b]	B11	2		40		
" "	1637				17	18
" "	1875					31
" Valentinum[b]	B15			2	13	
" vexillo-calyculatum Kellogg	606					17
" virga A. Gray	613	6				

[a] See Table 1 for explanation of reaction.

[b] Received under this name from E. Baur. See Zeitschrift für Induktive Adstammungs- und Vererbungslehre 63: 256–302. 1932.

[c] Received from E. Baur under designation A. meonanthum × Schw. B.

The marked resistance found in some of the species suggested the desirability of attempting crosses with commercial types of snapdragon. In 1927 resistant plants of *Antirrhinum glutinosum* 200 were crossed with the resistant strain, G W D1, of Giant White. The plants of the first generation

were all extremely resistant. The second generation produced 11 very susceptible, 40 moderately resistant, and 45 highly resistant plants. It was possible to continue only a limited number of the most promising resistant selections and these continued to give segregations into various susceptible and resistant types for the next 2 years. In 1932, 2 cases (9–1–3 and M12) of clear cut separation into an extremely resistant and a very susceptible group were obtained. The next year 5 lines proved to be homozygous for marked resistance. Usually there is no sign of infection when the plants are inoculated, although occasionally a few minute uredia may be produced. These lines in general, resemble the Giant White parent. The flowers are white but somewhat smaller than those of Giant White. They also show a tendency towards a narrow and somewhat pointed standard, characteristics derived from *Antirrhinum glutinosum*. While the writer considers that this has a certain grace as well as novelty, it is a departure from the standard and will probably not meet with the approval of the florist.

In 1932 several crosses were made with the most outstanding resistant segregates of 9–1–3 lines, using susceptible commercial types for the other parents. As stated above, the resistant lines were still segregating and, in the F_1, 2 of the crosses produced highly resistant and very susceptible segregates in ratios of 10:11 and 12:14. One produced only highly resistant plants. The F_2 from 3 resistant plants of the F_1 gave the following ratios, closely approximating 3 highly resistant and 1 very susceptible. The results obtained were as follows:

	Resistant	Susceptible	Deviation	$\frac{\text{Dev.}}{\text{P.E.}}$
	109	40	2.75	.77
	83	36	6.25	1.96
	98	25	5.75	1.77
Total	290	101	3.25	.56

From this it is evident that the marked resistance of the 9–3–1 lines is a simple dominant factor. White (16) reports obtaining similar ratios in crosses using resistant plants from early selections in this material. He apparently has been successful in developing several highly resistant commercial types.

Among others a species labeled *Antirrhinum Ibanjezii* was received from E. Baur in 1932. As shown in table 2, this species proved to be highly rust resistant. It was crossed with a susceptible commercial pink snapdragon. The F_1 generation was uniformly (21 plants) highly resistant, similar to the *A. Ibanjezii* parent. The F_2 segregated in a ratio of approximately

3:1, giving 113 extremely resistant and 32 very susceptible individuals, a deviation of 4.25 and Dev./P.E. of 1.20. Here, also, resistance is apparently due to a simple dominant factor.

DISCUSSION

Puccinia Antirrhini, although first collected on snapdragon, *Antirrhinum majus,* and described from that species, apparently was originally endemic to the Pacific Coast of North America on various species of Antirrhinum. Of the 12 species listed by Jepson for California, 5 have been studied in these investigations. *Antirrhinum maurandioides* and *A. virga* proved to be highly resistant. *A. Nuttallianum* contained highly resistant, moderately resistant, and susceptible individuals. *Antirrhinum glandulosum, A. Coulterianum,* and *A. vagans* (*A. vexillo-calyculatum* according to Munz) were susceptible. Arthur lists *A. Nuttallianum* and *A. virga* as hosts for the rust in California. *Antirrhinum majus* is native to the Mediterranean region where the rust is unknown. The marked susceptibility and the rarity of resistance in *Antirrhinum majus* also might be considered as further evidence, since previous to introduction into California there would have been no opportunity for selection of resistant strains. However, this alone would not be sufficient, since it is interesting to note that other exotic species, such as *Antirrhinum glutinosum, A. hispanicum, A. molle,* and *A. siculum,* show fully as much or more resistance than the California species of Antirrhinum.

In these studies only species of Antirrhinum have been successfully infected. All the species of Linaria and Adenostegia investigated have proved resistant. The resistance of *A. pilosa* raises some interesting questions. Based on the telia collected on *A. pilosa* (*Cordylanthus pilosus*) Arthur (1) described the species *Puccinia Adenostegiae.* Later, Blasdale (4) proposed the species *Puccinia Cordylanthi* for rust collected on *Cordylanthus filifolius* (*Adenostegia filifolia*). Arthur (2) considers these as synonymous with *Puccinia Antirrhini,* thus adding *A. filifolia, A. pilosa,* and *A. rigida* as hosts for the species. The results reported here suggest that the rust on species of Adenostegia may be distinct, at least so far as its host specialization is concerned. However, since only one collection of *Adenostegia pilosa* was studied, it may be that a resistant strain of the species was employed. Further study with other collections of the species of Adenostegia and also with rust from Adenostegia on Antirrhinum is necessary.

The observations and studies of most investigators have indicated that the species *Antirrhinum majus,* as represented by cultivated varieties, is very susceptible. Only Doran (7) has reported any pronounced differences among such varieties. His data are difficult to interpret. Under the heading "Scale, relative numbers," he lists with the value 0 such varieties as

Queen of the North, Giant White, Queen Victoria, Mont Blanc, Rose Dore, and Firebrand, all of which, in the writer's studies, have been susceptible, under favorable conditions for rust development, being entirely or mostly killed. Doran (p. 62) states that "these varieties, while not absolutely resistant, are the nearest approach to it among snapdragons" and it would appear that he has employed a classification in which 0 is used as a designation for the least susceptible of the varieties studied by him.

In these investigations no pronounced differences have been noted between cultivated varieties when considered as a whole. Individual differences, however, have been noted within such varieties. In most instances these were not pronounced. Such individuals survived where the majority were killed by the rust. Although they were considerably rusted, the uredia were fewer and smaller than those of the more susceptible individuals. Self pollination and selection for resistance through several generations resulted in the segregation of highly resistant types in which little or no rust developed under the conditions most favorable for the rust.

No attempt was made to keep complete genetical data, since it was possible to devote only a limited amount of time to the problem. The development of sterility as inbreeding progressed also introduced complications and seriously interfered with the selection of lines homozygous for marked resistance. It seems evident that the original selections from the cultivated varieties carried factors for several types of resistance. One of these governing the development of the marked resistance has been isolated by Emsweller and Jones. They have shown that it is inherited as a simple dominant factor, and have successfully utilized it in developing highly rust-resistant commercial types.

It also seems evident that the pronounced resistance occurring in some of the wild species of Antirrhinum and possibly certain wild strains of *A. majus* can be successfully utilized in the development of rust-resistant snapdragons. From the cross, *A. glutinosum* × Giant White Selection, a highly rust-resistant type, approaching Giant White in plant and flower characteristics, has been obtained. The pronounced resistance of this strain is also inherited as a simple dominant factor. White (16), from this same cross, has been successful in developing several commercial types having pronounced resistance. The inheritance of the marked resistance of *Antirrhinum Ibanjezii* B5 as a simple dominant factor when crossed with susceptible commercial strains of snapdragon indicates that the resistance of this species can be similarly utilized.

While the results that have been obtained indicate that resistant strains of snapdragon will probably solve the rust problem, the possibility of physiologic forms in the rust and the complications that might result should not be overlooked. So far, there has been no certain evidence of the occurrence

of such forms. The development of homozygous highly resistant lines of snapdragon now makes it possible to approach this problem with some degree of certainty. In this connection the aecial host also may play an important part. Recent investigations in the rusts all indicate that their sexual reproduction is connected with the aecial stage. The aecia, therefore, may play an important rôle in the development of physiologic forms. Elsewhere (12), it has been shown that *Puccinia Antirrhini* is heteroecious. So far, the aecial stage has not been discovered. In a rust of the type of *P. Antirrhini* it is difficult to foretell the alternate host. Since the rust is apparently native to California, the most promising method would be to locate heavily rusted California species of Antirrhinum and watch the species of other genera in the immediate vicinity for aecia, checking such observations with cultures.

SUMMARY

In these investigations only species of Antirrhinum have been infected with *Puccinia Antirrhini*. Species of Linaria and *Adenostegia pilosa* have been resistant.

The results obtained with species of Antirrhinum are summarized in table 2.

Cultivated varieties of *Antirrhinum majus* have, for the most part, been very susceptible. A few selections were obtained showing some resistance. From these by self pollination and selection, highly resistant types have been obtained.

Highly resistant types also have been obtained from crosses of snapdragon with resistant selections of *Antirrhinum glutinosum* and *A. Ibanjezii*. In each case, resistance has been obtained that is inherited as a simple dominant factor.

The resistance derived by selection from varieties of snapdragons and from the *Antirrhinum glutinosum* cross has been utilized in California by Emsweller and Jones (8) and in Massachusetts by White (16) for developing highly resistant commercial types of snapdragon.

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STUDIES ON THE HOST RANGE OF PHYTOMONAS STEWARTI AND P. VASCULARUM[1]

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INTRODUCTION

The host range of *Phytomonas stewarti* (E. F. Smith) Bergey *et al.*, the causal organism of Stewart's wilt disease of corn,[3] *Zea mays* L., was the subject of studies the results of which are reported in this paper. If hosts other than corn existed, they might be important in the life history of the disease. In order to determine whether some other species might be infected by the Stewart's wilt pathogen, a series of inoculations was made in the greenhouse and in the field upon the following plants: sorghum, *Holcus sorghum* L.; sugar cane, *Saccharum officinarum* L.; Sudan grass, *Holcus sudanensis* Bailey; yellow foxtail, *Setaria glauca* Beauv.; German foxtail millet, *Setaria italica* Beauv., var. *Stramineofructa* Bailey; and common millet, *Panicum miliaceum* L.

The number of bacterial pathogens that cause symptoms on corn similar to those caused by *Phytomonas stewarti* on corn also was investigated.

The similarity between the symptoms of Stewart's wilt and those of Cobb's disease of sugar cane,[6] prompted the writer to test the effect of *Phytomonas vascularum* (Cobb) Bergey *et al.* on corn and subsequently on sorghum.

MATERIALS AND METHODS

Unless otherwise stated, the culture of *Phytomonas stewarti* used in the inoculation tests was a progeny of a single cell. It was the standard culture employed by the writer throughout his previous studies on Stewart's wilt.[7]

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] The writer wishes to acknowledge his gratitude to Eugene Herrling for the photographs.

[3] The term "Stewart's wilt" disease in this paper refers to the disease caused by *P. stewarti* as described by Stewart[4] and Smith.[5] The term "bacterial wilt" is used to include Stewart's wilt disease and any other disease expressed by similar symptoms and caused by a bacterial organism or organisms other than *P. stewarti*.

[4] Stewart, F. C. A bacterial disease of sweet corn. New York Agr. Expt. Sta. Bul. 130. 1897.

[5] Smith, E. F. Bacteria in relation to plant diseases. Vol. 3. Washington, D. C. (Carnegie Inst. Wash. Publ. 27). 1914.

[6] Smith, E. F. See footnote 5.

[7] Ivanoff, S. S. Stewart's wilt disease of corn, with emphasis on the life history of *Phytomonas stewarti* in relation to pathogenesis. Jour. Agr. Res. 47: 749–770. 1933.

The cultures of *P. vascularum* were obtained from A. F. Bell of the Bureau of Sugar Experiment Stations, Australia. Their identity was confirmed by cultural and pathogenicity tests. All inoculations were made by means of needle punctures at the base of the plant by employing either a common needle or a plant inoculator.[8] In every inoculation test besides the inoculated plants there were about an equal number of controls. Some were punctured, while othere were left untreated. In all cases both kinds of control plants remained normal and, in the few reisolation tests made, yielded no pathogenic bacterial organisms.

Sugar-cane cuttings of 2 varieties, Co 281 and CP 807, were secured from E. C. Tims of the Louisiana State University. Sorghum seed of the variety Roxorange (Waconia Orange) was secured from A. H. Wright, of the Wisconsin Agricultural Experiment Station. Seed of other sorghum varieties including Blackhull kafir, Darso, Dwarf Yellow milo, Kansas Orange sorgo, Gooseneck, and Red Amber sorgo were obtained from A. L. Clapp, of the Kansas State College of Agriculture and Applied Science. Sudan grass seed of an unknown variety was obtained from a commercial firm. The seed of the Yellow foxtail, common millet, and German millet was obtained from A. L. Stone, of the College of Agriculture, University of Wisconsin. Two varieties of corn were used, Golden Bantam and Golden Gem, both known to be very susceptible to Stewart's wilt.

Isolations of *Phytomonas stewarti* from the tissue of the inoculated plants were made in the usual manner, employing nutrient dextrose agar. The pathogenicity of all isolated cultures was tested by inoculations on corn. For culturing and isolating *P. vascularum,* Wilbrink's medium was ordinarily used, which had the following composition: sucrose, 20 gr., peptone 5 gr., K_2HPO_4 0.5 gr., $MgSO_4$ 0.25 gr., agar 17 gr., and water to make one liter. The medium was adjusted to pH 7.0.

The inoculation tests were started in the greenhouse in the fall of 1933 at Madison, Wisconsin, continued in the field during the summer of 1934, and in the greenhouse at Madison, during the fall of the same year.

INOCULATION TESTS WITH PHYTOMONAS STEWARTI

Corn. The leaf symptoms induced on corn have been described in detail elsewhere.[9] For the purpose of comparison some of these symptoms are illustrated in figure 1, A and B.

Sorghum. In the greenhouse the first symptom appeared about 5 days after inoculation as a reddening of the infected veins (Fig. 1, C). This was followed by the appearance of red semicircular or irregularly shaped areas on either side of the affected veins (Fig. 1, D and E). These areas in

[8] Ivanoff, S. S. A plant inoculator. Phytopath. 24: 74–76. 1934.

[9] Ivanoff, S. S. See footnote 7.

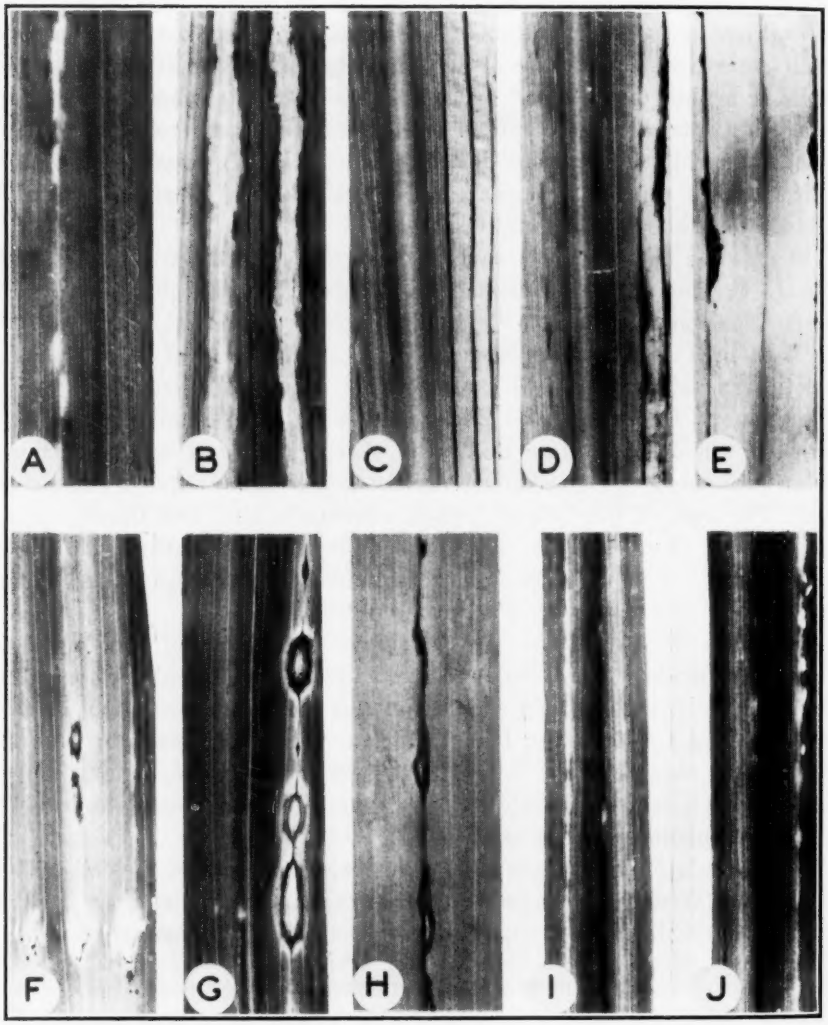

FIG. 1. Leaf symptoms on various plants induced by *Phytomonas stewarti* (magnifications approximate). A. Early stage on corn. ×1. B. Later stage of A. ×1. C. Early stage on sorghum. Red bundles appear black. ×1.5. D. Late stage of C showing invasion of the parenchyma. ×1.5. E. Later stage of D showing invaded parenchyma, red, sharply defined. ×3.5. F. Early stage on Sudan grass (holes at lower end from inoculation). ×1.5. G. Later stage of F. ×1.5. H. Stripe on German millet. ×3. I and J. Stripes on yellow foxtail. ×3.

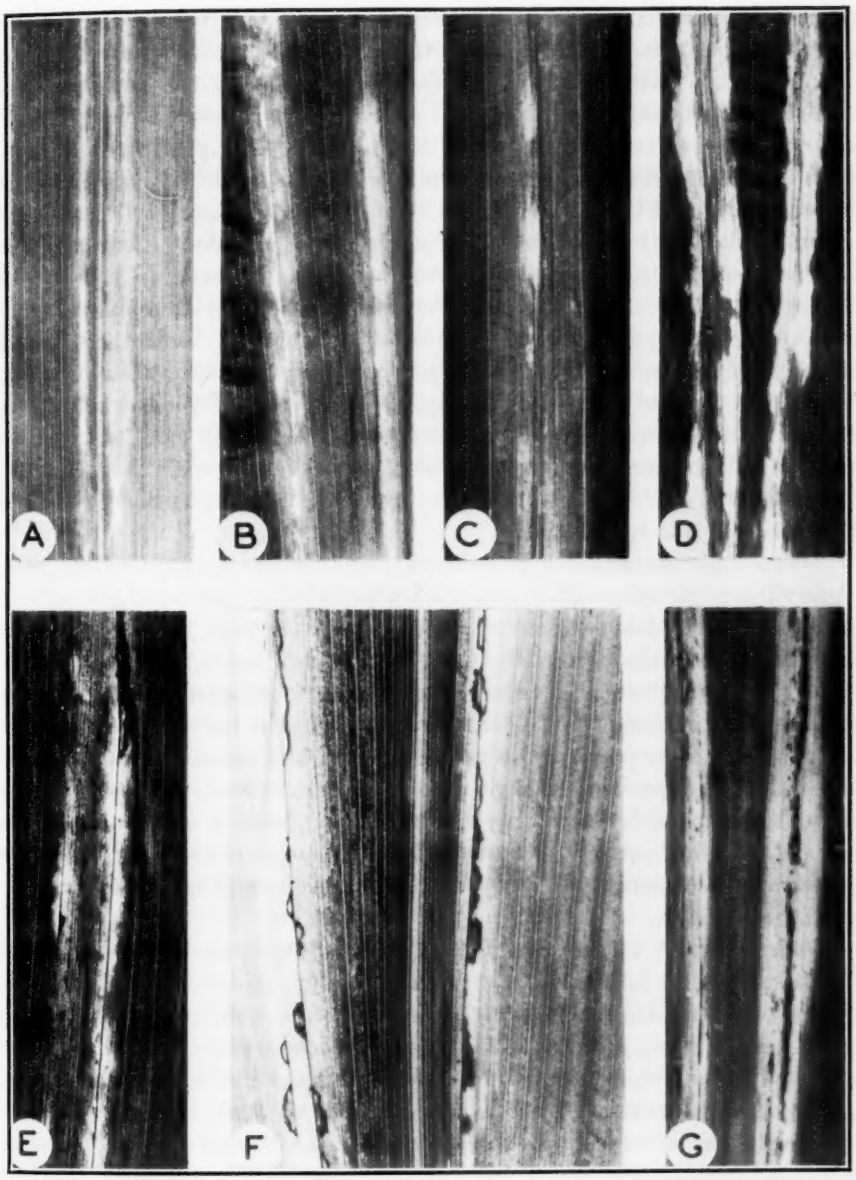

FIG. 2. Leaf symptoms on various plants induced by *Phytomonas vascularum* (magnifications approximate). A. Early stage on corn. ×1. B. Later stage of A, showing semitransparent water-soaked areas in the parenchyma. ×1. C. An enlarged view of semitransparent water-soaked areas along the veins of corn. ×3.5. D. Later stage of B. ×1. E. Early stage on sorghum. ×1.5. F. Later stage of E, showing characteristic red, semicircular areas along the veins. ×2.5. G. Stripes on sugar cane. ×1.

most cases were sharply delimited (Fig. 1, E), but occasionally the red pigment seemed to have diffused into the green tissue without leaving any definite demarcation (Fig. 1, D). The tissue adjoining the sharply delimited red areas usually lost its normal green color and became pale green to yellow. In some cases the inoculations caused wilting of entire leaves. Such symptoms were induced on plants belonging to all the varieties mentioned earlier and by more than one strain of the Stewart's wilt organism.

In the field only 2 varieties of sorghum were inoculated: Roxorange and Kansas Orange sorgo, the former being earlier and shorter in height than the latter. Over 1000 plants were inoculated twice, the first time about 5 weeks after planting and the second time about 7 weeks. Both the standard culture of *Phytomonas stewarti* and a mixture of strains, respectively, were employed. No leaf symptoms developed as a result of these inoculations. The stalks of Roxorange sorghum, however, developed red bundles that were plugged with bacteria and, in a few cases, formation of small cavities in the pith was observed. Kansas Orange sorgo, with a few exceptions, remained unaffected by the inoculations.

Microscopic examination of leaves that had become diseased in the greenhouse showed the affected bundles filled with bacteria that oozed out in large masses when these bundles were placed in contact with water. On either side of these bundles bacterial pockets were found that corresponded to the semicircular and irregularly shaped areas mentioned earlier. These pockets, like those in corn,[10] doubtless appeared where the bacteria escaped from the vessels into the parenchymatous tissue. In the manner of development and in shape, these abnormal areas on sorghum resembled these on corn. The outstanding difference between the leaf symptoms on sorghum and those on corn was the light red color of the infected sorghum tissue. The stripes on corn were semitransparent by transmitted light and tan to light brown by reflected light.

Sudan Grass. Two series of field inoculations were made on Sudan grass. The first series of inoculations were made on approximately 300 5-week-old plants with a mixture of 5 cultures, including the standard culture. As a result, only a small percentage of the plants showed any symptoms. These consisted in the reddening of the invaded vascular bundles of the leaves and stem and of a discoloration of the parenchymatous tissue along the infected leaf veins, similar to that in the case of corn. Although the bacteria were reisolated from several plants these symptoms were not considered striking. The second series of inoculations were made later in the season on about 100 3 to 4-week-old Sudan grass plants of a later planting with the standard culture. Disease symptoms developed on each of the inoculated plants but not on the controls. The symptoms that developed upon the leaves (Fig. 1, F and G) resembled those induced on sorghum by the red color of the

[10] Ivanoff, S. S. See footnote 7.

stripes and by the discoloration of the parenchymatous tissue along the affected veins. In many cases 2 of the semicircular areas occurring on both sides of a vein had a common center that resulted in the formation of elliptical spots along the vein (Fig. 1, G). The central portion of these spots in time became necrotic and turned light brown in color. In some cases the dry necrotic tissue fell out and left an elliptical hole in its place (Fig. 1, G). About 3 weeks after the inoculation, some of the stems were cut open and were found to contain some red bundles from which the bacteria were reisolated.

Common and German Millets. Most of these inoculations were made in the greenhouse with the standard culture of *Phytomonas stewarti* and with a mixture of strains of the same organism. The stripes that developed upon the leaves resembled in color, shape, and manner of development, more closely those produced on corn than those on any other plant tested. The only important difference was in the relatively narrow width of the stripes on millet, which probably was because of the narrower host leaves (Fig. 1, H).

Yellow Foxtail. The leaf stripes induced on this host were characterized by being very narrow, well defined, and a dull white or tan color (Fig. 1, I and J). Although no well defined semicircular, water-soaked areas along the veins have been observed to precede the formation of these stripes, the evanescent quality of such areas may have been missed because of the smallness of the leaves.

Sugar Cane. About 25 sugar-cane plants were inoculated in the greenhouse with 2 strains of the Stewart's wilt organism when the plants were 4 to 5 weeks old. Although the results were negative, the extent of the trials does not justify the conclusion that sugar cane is immune.

Cross inoculations were made by using the organism reisolated from sorghum upon corn, Sudan grass, and the millets, and *vice versa.* No change in the pathogenicity of the bacteria was observed as a result of the passage through other plants.

The existence of hosts to *Phytomonas stewarti,* other than those already named, is thought to be very probable.

The natural occurrence of the disease upon these hosts in the field has not yet been observed by the writer. An important reason may be the fact that during the summer of 1934 the incidence of Stewart's wilt on corn in northern Illinois and Wisconsin, where these observations were made, was negligible. On the most susceptible open-pollinated varieties of corn the disease ranged from 0 to a fraction of 1 per cent.

INOCULATION TESTS WITH PHYTOMONAS VASCULARUM

Greenhouse inoculations on corn and sorghum with *P. vascularum* revealed 2 things: firstly, that this microorganism causes disease upon these

plants, and secondly, that the symptoms induced by it are so similar in appearance to those induced upon the same hosts by *P. stewarti* that, so far, they have been differentiated with difficulty, if at all.

Corn. Three types of symptoms were induced by inoculating corn with *Phytomonas vascularum:* water-soaked stripes along infected leaf veins, cavities in the stalk, and general stunting.

The leaf stripes (Fig. 2, A–D) developed just as did those caused by *Phytomonas stewarti,* with this exception, that the incubation period in the greenhouse in the case of *P. vascularum* was about twice as long as that of Stewart's wilt. In addition, the water-soaked areas induced by the sugarcane organism assumed in time a somewhat rusty color, while those of Stewart's wilt were light brown. A third difference was the appearance of numerous, small, circular, elliptical, or irregular, water-soaked areas in the parenchymatous tissue of the leaf, away from the vein infected by *P. vascularum* (Fig. 2, B), and their absence in the case of *P. stewarti.* In each case these differences in the leaf symptoms caused by the 2 species of bacteria could be observed only at the right time during the course of their development and upon close examination. A glance at the respective symptoms at a late stage of development would commonly fail to differentiate them.

Cavities in the stalk of Golden Gem sweet corn were observed in some cases about 5 weeks after inoculation with *Phytomonas vascularum.* The development of these cavities started with the appearance of water-soaked tissue at the terminal bud of the culm and at the ear bud on the side of the culm (Fig. 3, G). Later the water-soaked tissue became very soft, changed to light brown, then to dark brown. Then the cavity appeared, apparently as a result of the digestion of the tissue by the bacteria (Fig. 3, H). The size of these cavities enlarged in length and width with the elongation of the stem internodes. In development and appearance these cavities resembled those induced on the variety Leaming of field corn inoculated in the field with a strain of *P. stewarti* (Fig. 3, A–D). In addition to the organism introduced, some other bacteria were isolated later from these cavities in great abundance. The effect of these secondary organisms on the size and consistency of the rotted tissue in the stalk remains to be investigated both in the case of *P. vascularum* and of *P. stewarti.*

Dwarfing and stunting of Golden Gem corn plants inoculated with *Phytomonas vascularum* probably is due to the activities of the bacteria in all of the infected tissue and particularly because of the cavities previously mentioned. Inoculated plants that developed leaf symptoms but did not develop cavities appeared only slightly smaller than the punctured controls. On the other hand, plants that developed cavities were considerably smaller than the control plants and their nodes were much closer together than were those of the controls.

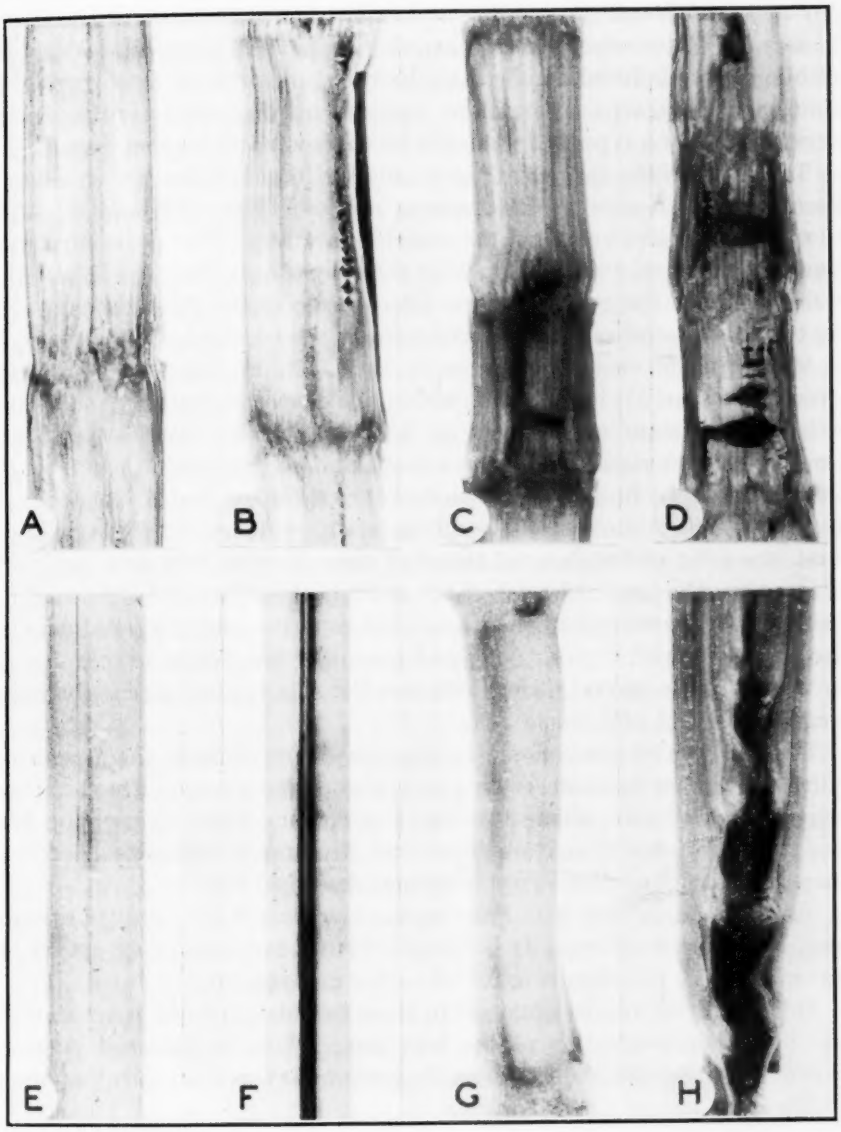

FIG. 3. Stalk rot of corn and sorghum induced by *Phytomonas stewarti* and *P. vascularum*. A–D. Four stages in the development of stalk rot of Leaming field corn, induced by a strain of *P. stewarti*. Color of cavity wall light to dark brown. ×1. E–F. Two stages in the development of stalk rot of sorghum induced by *P. vascularum*. Color of infected tissue deep red. ×2 and ×1.5, respectively. G–H. Two stages in the development of stalk rots of Golden Gem sweet corn, induced by *P. vascularum*. Color of infected tissue light to dark brown. ×1.5.

Sorghum. Roxorange sorghum plants, when inoculated in the greenhouse with *Phytomonas vascularum,* developed leaf symptoms closely resembling those induced on the same host and on corn by *P. stewarti.* In addition the sugar-cane organism induced rotting and cavities in the sorghum stalk of a type that seemed to be characteristic for that host.

The leaf stripes, in general, were similar in shape but not in color to those induced on corn by *Phytomonas stewarti* (Fig. 2, E and F). The color of the infected veins and the semicircular areas in the parenchymatous tissue along the vein was red, making the stripes conspicuous. In addition to the red tissue there was the usual discoloration of the green tissue bordering the red areas where the bacterial pockets were located.

Reddening of vascular bundles in the stalk, rotting of the pith and formation of cavities in the stalk resulted in some cases from the inoculation with the sugar-cane organism (Fig. 3, E, F). These cavities had 2 outstanding characteristics in color and shape that distinguished them from the cavities produced in corn by *Phytomonas vascularum* and *P. stewarti* and from the small pockets in the sorghum stalk produced by *P. vascularum.* First, the color of the decayed tissue of these cavities was deep red, more intense than the light red color of the small pockets formed in the sorghum stalk by *P. stewarti,* and second, these cavities in the early stages of development were straight, regular, long and narrow. They extended throughout the length of the several nodes, with parallel side borders strikingly delimiting the affected pith tissue (Fig. 3, F).

For the sake of comparison, inoculations were made in the greenhouse with *Phytomonas vascularum* on young sugar-cane plants. The usual leaf stripes appeared after about 2 weeks (Fig. 2, G). These stripes have been described previously[11] and are illustrated here for convenience in making comparisons with similar stripe symptoms described above.

The inoculation tests with *Phytomonas vascularum* were limited to sugar cane, corn, and sorghum. It is likely that further study may extend the host range of *P. vascularum* to include other grass plants.

In view of the results obtained in these inoculation tests, it seems probable that an investigation of the host range of other bacterial parasites known to induce stripe symptoms on grasses may reveal other important relationships among these parasites.

DISCUSSION

For the sake of clarity the foregoing results have been rearranged as follows:

The symptoms induced upon the leaves by either of the 2 pathogens on any of the hosts mentioned had some common resemblances and some individual distinguishing characteristics, depending upon the host.

[11] Smith, E. F. See footnote 5.

The similarity features of these symptoms were the formation of stripes along the veins in a characteristic manner, already described in the case of Stewart's wilt of corn,[12] namely, by the change of color of the affected vein for some distance above and below the point of entrance of the bacteria; by the appearance of semicircular or irregularly shaped areas on either side of the vein, with a consistency and color depending upon the host; and by a general discoloration of the green parenchymatous tissue adjoining these areas.

The differences thus far noted as to the appearance of the leaf symptoms in the various hosts consisted in the color of the veins and of the semicircular or irregular areas adjoining the affected veins, in the consistency of the tissue of these areas, in the arrangement of these areas, and in the duration of the incubation period.

The color of the affected veins and that of the semicircular or irregular areas of plants inoculated with *Phytomonas stewarti* was tan to light brown in the case of corn, yellow foxtail, and the millets, and red in the case of sorghum, and Sudan grass. The color of the same infected tissue of plants inoculated with *P. vascularum* was light brown to rusty in the case of corn and red in the case of sorghum. The consistency of all light brown areas, just mentioned, in the case of all hosts and both pathogens, was at first water-soaked. These areas later became necrotic and dry. The red areas in all hosts did not appear water-soaked probably because of the obscuring effect of the red pigment.

The arrangement of the semicircular or irregular areas adjoining the affected veins was, in all cases but one, in a more or less alternating manner. In the case of Sudan grass inoculated with *Phytomonas stewarti,* very often two of these areas occurred opposite each other, thus forming a more or less round or elongated spot that had its center at the vein.

The incubation period in the case of corn inoculated in the greenhouse with *Phytomonas vascularum* was twice as long as in the case of the same host inoculated with *P. stewarti.*

Rotting of the corn and sorghum stalk tissue in various degrees was produced by *Phytomonas stewarti* and *P. vascularum.* The affected sorghum tissue developed a red color of different intensities, according to the pathogen introduced. In the case of *P. stewarti* the color of the diseased tissue was light red; in that of *P. vascularum* it was deep red. No striking differences thus far have been observed in the stalk rots produced on corn by the 2 pathogens.

The most favorable stage of host development for infection and the comparative susceptibility of the various hosts to both pathogens have not been determined.

[12] Ivanoff, S. S. See footnote 7.

SUMMARY

Symptoms are described that were induced by field and greenhouse inoculations with strains of *Phytomonas stewarti* on corn, *Zea mays;* sorghum, *Holcus sorghum;* Sudan grass, *Holcus sudanensis;* yellow foxtail, *Setaria glauca;* German foxtail millet, *Setaria italica* var. *stramineofructa;* and common millet, *Panicum miliaceum,* and induced by greenhouse inoculations with *P. vascularum* on corn, sorghum, and sugar cane, *Saccharum officinarum.*

The leaf symptoms induced by the Stewart's wilt organism on sorghum, Sudan grass, yellow foxtail, German and common millets, with a few exceptions noted, resembled in shape and manner the development of those induced by the same pathogen on corn.

The sugar-cane organism induced symptoms upon the leaves of corn and sorghum that resembled those induced on the same hosts by Stewart's wilt pathogen.

The sugar-cane organism, in addition, induced a rotting of the corn stalk similar to that induced by *Phytomonas stewarti* on the same host. *P. vascularum* also induced cavities in the sorghum stalk.

NEW ECONOMIC HOSTS OF THE STEM- AND BULB-INFESTING NEMATODE

G. H. GODFREY AND C. EMLEN SCOTT[1]

(Accepted for publication January 21, 1935)

Steiner and Buhrer,[2] Cobb and Steiner,[3] and Goodey (5) have listed the hosts of the stem- and bulb-infesting nematode, *Anguillulina dipsaci* (Kühn) Gervais and van Beneden, and have shown a known host range of some 240 species of plants, about 30 of which are cultivated food plants. The present paper gives an account of the occurrence of this nematode on 3 hitherto (4) unreported food-plant hosts: salsify, *Tragopogon porrifolius* L.; parsley, *Petroselinum hortense* Hoffm.; and celery, *Apium graveolens* L. The first of these belongs to the family Compositae, the others to the Umbelliferae.

HISTORY OF OCCURRENCE AND SYMPTOMS OF THE DISEASE

In April, 1933, specimens of diseased salsify plants were received by the Division of Plant Pathology, University of California at Berkeley, from the vicinity of San Juan, San Benito County. Symptoms suggested stem- and bulb-nematode infestation, and microscopic examination verified this diagnosis. The nematodes were found in great abundance in swollen leaf bases, in young leaves just issuing from the crown, and here and there in swellings in leaf blades. The infected tissues in the crown region were cream color, becoming brown, instead of the normal white and light green. In tissues showing advanced stages of injury, some necrosis occurred, followed by secondary organisms. Nematodes were present in all stages, eggs, larvae of different ages, and adults. Measurements of adult males and females were typical for the species as described by Cobb (1) and in great detail by Goodey (5).

In late April the writers visited the field whence the specimens had come, one of many there devoted to seed production. The well-established salsify plants had been transplanted from a seed bed in the fall of 1932, and their flower stems were just beginning to shoot. The field had been devoted to

[1] Grateful acknowledgment is made to Professor W. B. Herms, Division of Entomology and Parasitology, University of California, for materials and equipment used in connection with the studies herein reported, and for making possible the photographs used for illustrations.

[2] Steiner, G., and E. M. Buhrer. A list of plants attacked by *Tylenchus dipsaci*, the stem or bulb nema[illegible]. S. D. A., Bur. Plant Indus., Plant Dis. Rptr. **16**: 76–85. 1932.

[3] Cobb, Grace Sherman, G. Steiner and F. S. Blanton. Observations on the significance of weeds as carriers of the bulb or stem nematode in narcissus plantings. U. S. D. A., Bur. Plant Indus., Plant Dis. Rptr. **18**: 127–129. 1934.

garlic the year before, and, significantly, the bulb nematode was reported present in that crop. About 10 per cent of the salsify plant spaces were vacant, the plants having been rogued out and destroyed because of disease. An additional 5 per cent (estimated) had unmistakable symptoms of the nematode disease. Since other diseases, including a heart rot caused by *Sclerotinia libertiana* and a yellow dwarfing disease suggestive of aster yellows were present, it is probable that only a part of the failures noted were due to the nematode disease.

Adjacent to the salsify field were fields of garlic showing also incidence of nematode injury, ranging from 2 to 10 per cent in some areas. The

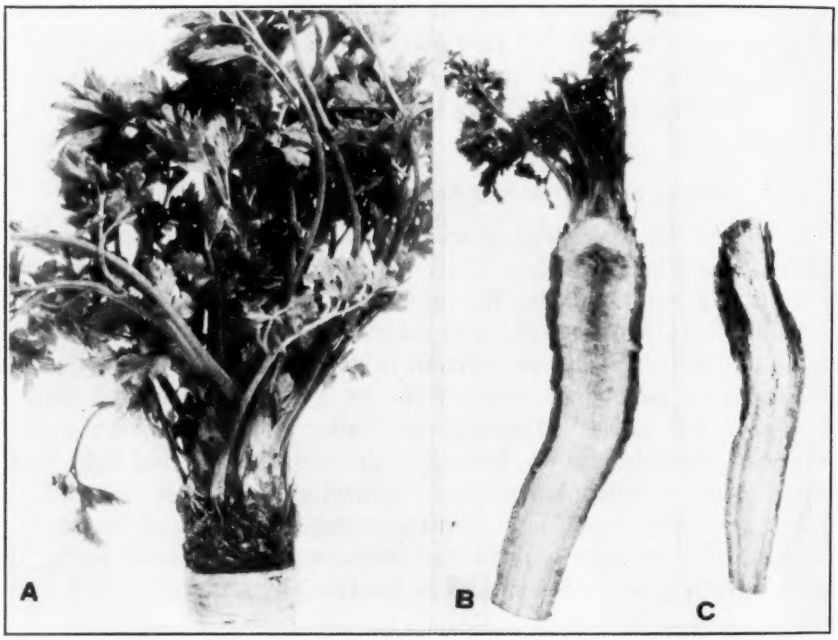

FIG. 1. A. A stem-nematode-infested Hamburg parsley plant, showing necrosis and shredding of leaf-petiole bases and yellowing of outer leaves. Leaf petioles showing such symptoms are interiorly discolored and nematode invasion extends even into the leaflets. ×¾. B. and C. Hamburg parsley roots showing typical symptoms of stem-nematode attack. B. Invasion probably directly from the soil and extending into the central part of the root. C. Invasion probably in part, at least, from above, for complete necrosis had occurred in the crown region. In both the nematodes extend considerably beyond the visibly infested portions.

variety known as Late garlic was much more highly susceptible than Early garlic, though diseased plants of the latter, definitely in evidence, were marked by a progressive yellowing or browning of lower leaves. Close examination disclosed unmistakable symptoms of nematode invasion, *i.e.*,

thickened scales at the leaf bases, sometimes with splitting, and eventual necrosis in the more severe cases. It was to be noted that no "spikkels" or swollen areas of yellowish coloration occurred on the leaf blades, such as is seen in Narcissus. Subsequent laboratory examination showed the nematodes to be exceedingly abundant in the yellowed leaves, in the bulb scales, and even in green leaves showing no obvious symptom. The occurrence of this nematode in garlic and onions is well known.

A near-by parsley field also showed serious nematode injury, which was even more pronounced than in the salsify. An average of 10 per cent (rough estimate) of the plants were clearly affected. The symptoms resemble those caused by the same nematode in alfalfa and clover, *i.e.*, dwarfing of leaves and leaflets, "tulip-root" effect in leaf petiole bases, distorted regions in the leaves; though, in general, this macroscopic symptom so common with other hosts was conspicuously rare. In severer cases leaf petiole bases were split and a brownish necrosis in the interior region was evident.

The most striking case of the disease ever noted by the writers in any crop was found in a field of Hamburg parsley, some 5 or more acres in extent, about a mile distant from the first field. This, too, had been planted for seed production. At first glance it was evident that more than 50 per cent of the plants were missing. Many of those remaining were dwarfed and off-color, and still others showed the leaves wilted to the ground. Mildly affected plants showed symptoms similar to those described above for plain parsley. Figure 1, A illustrates a typical specimen, with petiole bases swollen and split, brown within, and outer leaves turning yellow. More extreme cases showed severe necrosis at the point of attachment of the leaves. When attempts were made to pull such plants for examination, the leaves would break off, leaving the roots in the ground.

Root Symptoms in Parsley. Such roots, when removed, showed necrotic regions extending well into the roots. In some cases such regions distinctly extended downward from the crown, and frequently the central region as well as the cortical region of the roots was so affected. In other cases, as shown by sectioned roots, cortical necrosis occurred apparently independently of the crown. It was not until such roots were examined in the laboratory that it was definitely determined that the discoloration in roots was accompanied by extremely heavy invasion by nematodes. These organisms appear to have found a highly favorable medium for growth and reproduction in the parsley roots, invading even white portions of the root beyond the evident discolored region. The evidence indicated that invasion of roots frequently took place directly from the soil and was quite independent of leaf invasion. Figure 1, B and C, shows 2 parsley roots, the first with independent root invasion and the other with the greater part of the injury apparently progressing downward from the crown.

Approximately a month after the first field observation, a second visit was made to these fields. It was then evident that the nematode disease was progressing rapidly in all diseased plants, but particularly so in the Hamburg parsley. An exact count was made in 4 representative 600-foot rows of this crop and it was determined that almost exactly 80 per cent of the stand was either missing (having been rogued out and destroyed) or dead. Of the remaining plants many were dwarfed and yellowed, and it was obvious that they, too, would perish before seed production. The field, therefore, was virtually a total failure.

OBSERVATIONS ON THE SUSCEPTIBILITY OF OTHER PLANTS

Adjacent to the Hamburg parsley were fields of salsify, Early and Late garlic, and double- and triple-curled parsley, all with occasional plants showing nematode infection, but much less than did the Hamburg parsley. Either this plant was distinctly much more susceptible than were the other plants, or the field in which it was planted was initially more heavily infested than were the surrounding areas. One other hitherto reported host, sweet pea, *Lathyrus odoratus* L., planted for seed production in a near-by field, likewise showed an occasional plant with typical nematode disease symptoms and definite nematode invasion. Orchard morning-glory, *Convolvulus arvensis* L., which grew as a weed in the most heavily infested areas, was apparently entirely free, although it has been reported as a host.

The occurrence of the disease in the third new host, celery, was not observed in the field, but rather in cultures of the plant in pots, following artificial greenhouse inoculations with colonies of nematodes from garlic and parsley, as described elsewhere in this paper.

THE CAUSAL ORGANISM

By way of substantiation of the identity of the nematode, measurements according to Cobb's formula are given of specimens taken from Hamburg parsley, as follows:—

Females, average of 14 adults,

.8	7.5	?	81.5	93	
.8	1.9	2.3	2.4	1.3	1177 μ

Males, average of 8 adults,

.8	7.5	?	M	93.8	
.8	1.8	2.1	2.2	1.5	1131 μ. Spicule, 22.1 μ

Even though the proportional distance to the intestine is omitted in both cases because of the obscurity at this point in the material examined, other measurements and anatomical details check so closely with accepted descriptions that there can be no doubt as to the identity. Random measurements from the other newly reported hosts checked equally well, and are not reported.

LOCATION OF NEMATODES IN DISEASED TISSUES

In the course of laboratory examinations of diseased material it was found that nematodes in great abundance permeated tissues in which outward manifestation of the disease was evident. In addition, border tissues not showing injury, and sometimes tissues several inches away from obvious injury, were found to be swarming with nematodes in all stages of development. In garlic, unswollen leaves of normal color above infested scales contained hundreds of them (3). Dissection of parsley leaves showing swellings in petiole bases and internal discoloration disclosed the upward extension of the internal petiole browning, sometimes into leaflet petioles, and that such regions were invaded throughout by nematodes. More than this, the leaflets themselves were frequently invaded, even in the mesophyll. Figure 2, A shows a typical leaflet cleared in clove oil with the nematodes stained to show them in natural position, following a special procedure described elsewhere (3). Such leaflets showed no distortion. When heavily infested they were slightly yellowed, sometimes mottled, and, like the rest of the leaf and the entire plant, were somewhat undersized. The path of leaflet invasion was undoubtedly the interior region of the leaf petiole, and midrib, though the latter showed no browning or distortion. Figure 2, B, a 1-millimeter section of infested Hamburg parsley root, similarly prepared, shows the extremely heavy invasion and reproduction that occur in this region of the plant. This heavy root infestation is unusual for the stem- and bulb-infesting nematode. The nematodes apparently migrate very readily through the tissues; they are found in abundance in the advancing margin of visible infestation, and even in the healthy white tissues just beyond. Occasional roots showed infestation within the central region, apparent invasion having occurred through the stele from the cortical tissues.

No plants of any of the hosts herein reported were seen in the process of seed development. It is therefore impossible to say whether or not seed invasion occurs, such as has been reported for *Hypochaeris radicata* and *Taraxacum officinale* and other hosts (2, 6). In garlic, where propagation is largely by means of bulblets (cloves), it is obvious that much spread by this means might readily occur.

EXPERIMENTAL INOCULATIONS

A simple experiment was conducted to determine in part the relationship of the particular strain of *Anguillulina dipsaci* found in these hosts, to other known strains. Only one other certain stem nematode strain, that in alfalfa, was immediately available. It was acquired in typical diseased plant materials from the Agricultural Commissioner of San Benito County. Another nematode, described tentatively by Steiner and Scott (7) as *Anguillulina dipsaci* var. *amsinckiae*, was collected in abundance in the galled seed heads

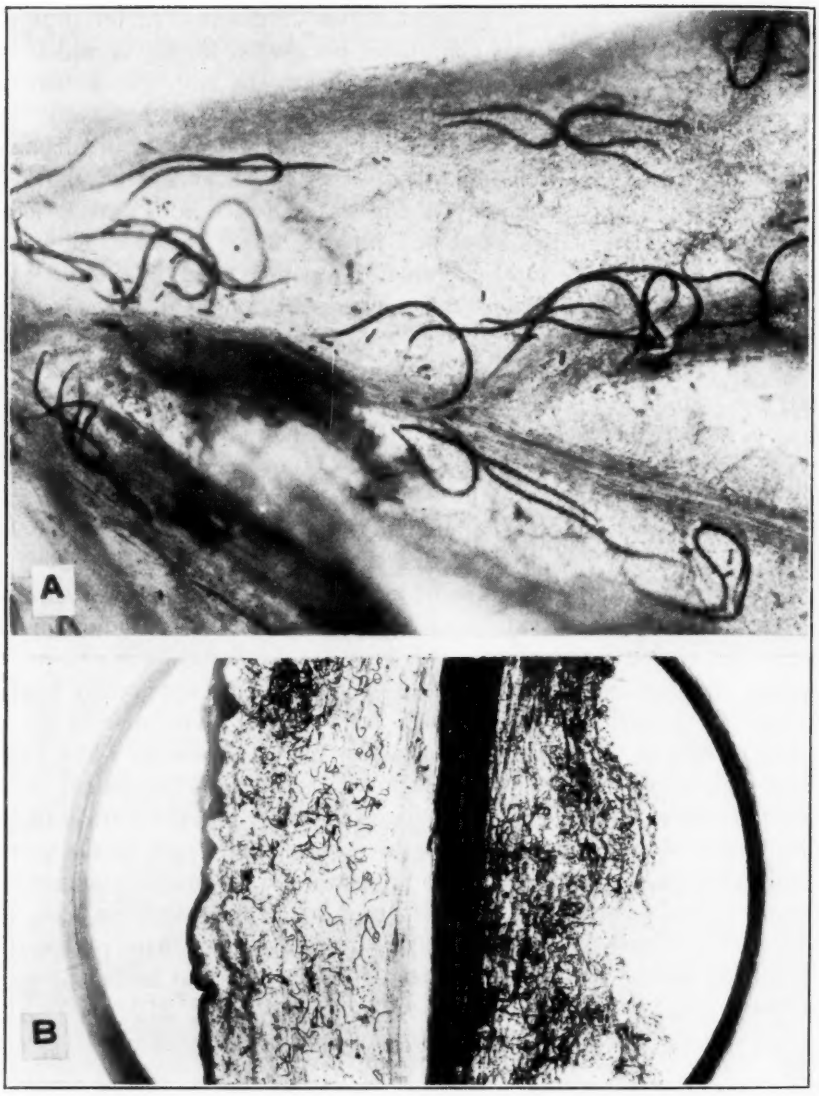

FIG. 2. A. Portion of a leaflet of Hamburg parsley (*Petroselinum hortense*) invaded by stem nematodes (*Anguillulina dipsaci*) without causing distortion. Eggs, larvae, and adults are present mostly in the spongy parenchyma of the leaf mesophyll. × about 30. B. A section about 1 mm. thick from a root of Hamburg parsley invaded by stem nematodes. Both the cortex and the central region are invaded. × about 6.

of fiddle-neck, *Amsinckia intermedia* F. and M., from the roadside south of San Jose, and used in the inoculations. These 2 strains, then, with the nematodes from garlic and from parsley, made 4 strains of the organism that were used to inoculate 4 series of large pots of soil. Rather heavy inoculations consisting of broken bits of fresh heavily infested plant materials, were applied in every case by stirring them into the upper 2 inches of soil. In each series were planted seeds of alfalfa, celery, parsley, salsify, and Amsinckia, and bulblets of garlic. The pots were kept moist through several months and frequent observations were made. The Amsinckia seeds were collected from the current season's growth and remained dormant, so no readings were possible on that plant. All other plants grew well. Final conclusions from the experiment are given in table 1.

TABLE 1.—*Results of a cross-inoculation experiment with Anguillulina dipsaci obtained from different sources*

Nematode sources	Presence or absence of infection in different plants tested				
	Alfalfa	Celery	Garlic	Parsley	Salsify
Alfalfa	+++	–	–	–	–
Garlic	–	++	+++	+++	++
Parsley	–	++	+++	+++	++
Amsinckia	–	–	–	–	–

Bur clover, *Medicago denticulata* Willd., listed by Goodey (6) as a host, which appeared as a weed in most of the pots, was likewise negative throughout the period of testing. It would appear that the garlic, parsley, and salsify strains were identical, all probably the original garlic strain of previous years; and that this was distinctly different from the alfalfa strain. It appears also that the Amsinckia strain or variety was distinct from either of the others. In this case an extremely high abundance of infective material was used throughout as inoculum, since every seed-head gall was literally swarming with larvae and dozens of galls were used in every pot. The celery plants, which showed positive infection by the garlic and parsley strains, showed symptoms similar to those in parsley, with swollen petiole bases, some necrosis, and invasion even of leaflets. Celery, parsley, and salsify, then, must be considered as inherently susceptible vegetables in any cropping scheme to follow nematode-infested crops of garlic.

NOTE ON THE LONGEVITY OF THE GARLIC NEMATODE IN SOILS

No experiments in this connection were conducted, but one observation seems worthy of record. The leading garlic grower in the vicinity of San

Juan showed us a garlic field in which the crop had been discontinued for a period because of the high incidence of the nematode disease. The field was devoted for a period of 6 successive years to a non-susceptible crop, lettuce. In the fall of 1932 garlic was again planted, and there was a healthy stand throughout. After careful searching, not a single nematode-infested plant was found. The 6 years in immune crops were apparently sufficient to eradicate the nematode from the field. It is very possible, of course, that a carefully conducted experiment with different rotation periods would show that a shorter period would suffice to do the same thing.

SUMMARY

Salsify, *Tragopogon porrifolius*, parsley, *Petroselinum hortense*, and celery, *Apium graveolens*, are reported as heretofore unrecorded food-plant hosts of the stem- and bulb-infesting nematode *Anguillulina dipsaci*. Symptoms in all alike were swollen leaf bases, "tulip root" effect in young shoots, yellowing and dwarfing of plants, and some necrosis in the crown region. Hamburg parsley was attacked particularly badly, a 5-acre field being practically a total failure. An additional symptom in this plant was a brown necrosis of the fleshy roots extending into both cortical and central regions. Invasion of leaflets, without distortion, was likewise demonstrated. The disease in salsify and parsley was found in plantings made for seed production in the vicinity of San Juan, San Benito County, California, in areas formerly in garlic, *Allium sativum*, in which the nematode disease was known to have been present. Diseased celery was not found in the field, but rather in cultures of this plant in pots following artificial greenhouse inoculations with colonies of nematodes from garlic and parsley.

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CULTURAL VARIATIONS OF THIELAVIOPSIS BASICOLA[1]

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Eleven cultures of *Thielaviopsis basicola* (Berk.) Ferraris, the causal organism of black root rot of tobacco, were isolated in June, 1930, by Gilbert's (3) method, from rotting roots of White Burley tobacco from 2 different fields on the Experiment Station farm at Lexington, Kentucky, where tobacco had been grown continuously for several years, and from a field in Woodford County, Kentucky. Seven cultures came from one field on the experiment station farm, 2 from the other, and 2 from Woodford County.

After 2 weeks of growth on potato-dextrose agar in tubes, the 11 cultures differed from each other in color and rate of growth. They were transferred when 2 weeks old to potato-dextrose agar in Petri dishes and kept for 2 weeks at 27° C. Each colony was uniform but most of the cultures differed in rate of growth, color, hyphal, and colony characters, and in spore characters. The 2 almost identical Woodford County cultures were dark olive (5)[2]; the aerial mycelium was deep grayish olive and powdery, and formed concentric rings. The experiment station cultures all differed and none resembled the Woodford County ones. The Station cultures were various shades of olive and brown and differed in appearance and rate of growth of the mycelium and in spore characters. Some cultures produced endoconidia almost exclusively, while in others chlamydospores predominated. One culture, which produced only chlamydospores, was subcultured 2 years and never was observed to produce endoconidia. When the 11 original cultures were 3 weeks old they were again transferred to plates of potato-dextrose agar and grown for 2 weeks at 27° C. The differences were more striking than in the first plating. Most cultures had at least one sector, some had 3 or 4, which differed from the rest of the colony. The variants appeared to have originated in the culture tubes, but became evident in the culture dishes.

SINGLE-SPORE CULTURES

The variations observed might have been due to mixtures in the original isolants. To eliminate this possibility, 26 single-endoconidium cultures were made from the original isolants by Ezekiel's (1) method. Two hyphal-tip cultures were made of the chlamydospore strains because of failure of chlamydospores to germinate readily on culture media. In the first plating

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] Ridgway color standards used in descriptions.

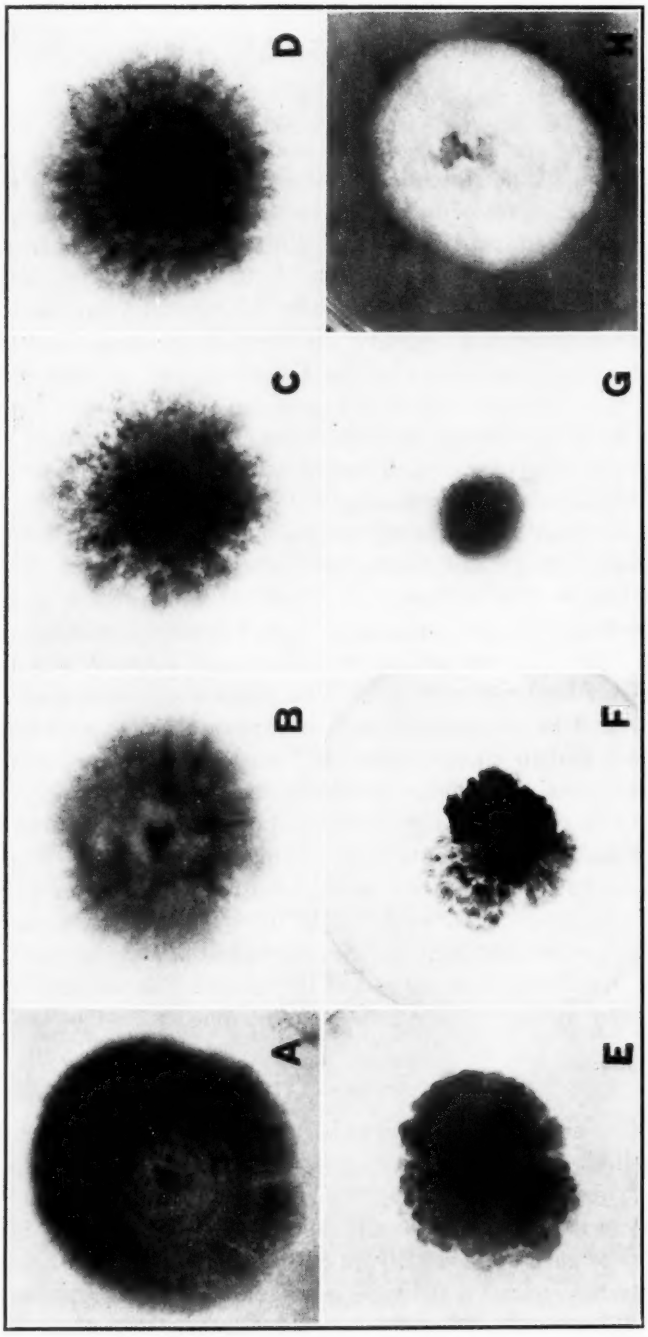

FIG. 1. Single-spore cultures of *Thielaviopsis basicola* after 11 days' growth on potato-dextrose agar. A and B. Same culture from different parts of the tube. C (224: 2: w), D (224: 2: y) and E (224: 2: z). From station farm. F and G. From Woodford County. H. Single-spore albino culture, which failed to develop color during 4 years' propagation.

on potato-dextrose agar 8 Woodford County single-spore cultures appeared almost identical and were similar to the original isolants from this field. Twenty station single-spore cultures differed from the Woodford County ones and from one another in color, rate of growth, hyphal characters, and in the ratio of the 2 kinds of spores. The station cultures were deep olive, dark olive, olivaceous black, plumbaceous black, cinnamon brown, and olivaceous brown. Some had abundant white and cottony aerial mycelium, while in others it was olivaceous gray and powdery, with variations between these extremes. In this plating very few sectors developed, the cultures having been in tubes for only 12 to 15 days. Seven single-spore cultures are shown in figure 1. In a second plating, made when the tube cultures were 27 to 30 days old, there were few cultures that did not have one or more sectors. The 2 hyphal-tip chlamydospore cultures, neither of which produced endoconidia, were strikingly different. One was dark olive with dense, white, cottony, aerial mycelium and had numerous large, 3- to 7-septate chlamydospores. The other was Dresden brown to Saccardo's umber; had dense, grayish, cottony, mycelium; and the chlamydospores were smaller and less numerous. The original 26 single-spore cultures and the hyphal-tip chlamydospore cultures, all grown in tubes 30 days, and subcultures from sectors in the previous plating, were again plated on potato-dextrose agar. So many culturally distinct sectors appeared that it was decided to confine further studies to 2 single-spore cultures from different sources.

Twenty-three plates of potato-dextrose agar were inoculated with a single-spore, dark olive culture (224:1:b, station farm) that had been in a tube for 60 days. Twenty-eight sectors that differed from one another developed. They were whitish, grayish, and various shades of olive and brown, and the aerial mycelium varied from dense and cottony through sparse and powdery to almost resupinate. Subcultures were made from all sectors that came to the margins of the colonies. These and the original single-spore culture (224:1:b) were again plated and subcultures were made from all sectors that appeared different. The subcultures were all found to be unstable and formed sectors. Sixty-six culturally distinct variants, which were deep, dark, grayish, ochraceous, and blackish olive, mummy, Vandyke, Sayal and Dresden brown, Saccardo's umber, sepia, ochraceous, cinnamon buff, clay, and white, developed in the 3 series of platings of single-spore culture 224:1:b. There were also variations in type of mycelium, rate of growth, colony, and spore characters.

The following example illustrates the apparent lack of a definite trend in color variation: From a single-spore dark olive culture (224:1:b) a whitish sector resulted in whitish and dark olive sectors; one of these whitish sectors produced dark olive and a Dresden brown sector. A grayish sector from the original dark olive culture resulted in brown and dark olive sectors. A deep olive sector from the first whitish sector gave a dark olive and an

almost white sector. A brownish sector from the original single-spore culture resulted in deep olive, grayish, and Dresden brown sectors. One of these grayish sectors gave an albino, a light brown, and a deep olive sector. With the exception of the albino sector, in which color production appeared to be lost completely and permanently, color changes from dark to light or from light to dark did not appear to be due in any case to permanent loss of a color factor.

Twenty-four plates of potato-dextrose agar were inoculated with 221: 1: b, a 60-day old single-spore, dark olive, tube culture (Woodford County). Only 4 culturally distinct strains developed; 2 olives, deep and dark, and 2 browns, Rood's and olive. There were differences in rate of growth, colony characters, and spore production. A few sectors formed in subsequent platings, but this culture was less variable than 224: 1: b.

SINGLE-SPORE CULTURES OF SINGLE-SPORE CULTURES

Thirteen single-endoconidium-spore cultures were made from 6 station single-spore cultures and from one Woodford County single-spore culture. In the first plating from 15- to 23-day-old tube cultures no sectors appeared, but in a second plating, when the tube cultures were 27 to 35 days old, many sectors formed. Thirteen more single-spore cultures were made from a 30-day-old tube culture, 224: 1: w, (Fig. 2, C) from which 224: 1: b, previously described, came. Eight of these cultures appeared alike, grew uniformly, were Dresden brown, and had numerous endoconidia and chlamydospores. The other 5 cultures differed from the 8 and from one another. Sectors developed in 4 of these (Fig. 2 E, F, G, H). When the 13 single-spore cultures of 224: 1: w were 28 days old they were plated in triplicate on potato-dextrose agar. These 28-day-old cultures showed greater variation than the 7-day-old cultures. Often, the colonies in 3 plates from one tube were culturally distinct. In the 39 plates, 15 sectors distinctly different from one another and from the original (224: 1: w), developed. Two more cultures that produced only chlamydospores developed in 2 sectors. So far, these and the chlamydospore culture isolated from rotting roots have not produced endoconidia, the factor for the production of which appears to have been lost. Tentatively, these cultures may be considered genetically different from the more complete type of organism from which they developed. This series of platings again suggests that the longer cultures grow in tubes the greater will be the number of variants when plated. Single-spore cultures seem to be as unstable as nonmonosporous cultures, although much additional work should be done to establish this point.

ALBINO CULTURE

An albino culture (Fig. 1, H) having both chlamydospores and endoconidia, obtained by subculturing a white sector from one of the station

FIG. 2. Cultures of *Thielaviopsis basicola* after 12 days on potato-dextrose agar. A (224: 1). Isolation from rotting tobacco roots; not a single-spore culture. B (224: 1: v), C (224: 1: w), and D (224: 1: z). Single-spore cultures from A. E (224: 1: w: 8), F (224: 1: w: 9), G (224: 1: w: 10), and H (224: 1: w: 16). Single-spore cultures from the single-spore culture shown in C.

cultures, has developed no color either in the hyphae or in the chlamydospores during 4 years of propagation. Seven single-spore cultures, from this albino, developed 3 culturally distinct colonies on potato-dextrose agar but all were white and had endoconidia and chlamydospores.

SPORE VARIATIONS

The cultures of *Thielaviopsis basicola* showed considerable variation in the number, kinds, and sizes of spores. In some, endoconidia predominated; in others, chlamydospores greatly outnumbered endoconidia. The complete absence of endoconidia in 3 cultures has been mentioned. No culture, having endoconidia only, was found in any of the tubes or plates. The endoconidia varied little in size from culture to culture, but there was considerable variation in the chlamydospores; some were borne in clusters of 3 to many; in others they were terminal and single; and in others single and intercalary. Some cells, which were spherical, hyaline, terminal or intercalary, and either solitary or in chains of three or more, developed in some cultures. These may represent another spore type.

HYPHAL CHARACTERS

In general the microscopic variations of the hyphae, in size and method of branching, were not great, being often as great within a culture as between cultures. One culture, however, developed, in addition to the normal mycelium, dark colored strands that readily broke into sections composed of single cells.

NO PERFECT STAGE OBSERVED

Plates were inoculated with different combinations of single-spore cultures, incubated at 27° C., and grown for some time after the hyphae intermingled, but no sexual fruiting bodies were observed. Many cultures were kept in tubes for a year, but no sexual fruiting bodies developed.

PATHOGENICITY TESTS

Eight single-spore cultures, including the albino and a hyphal-tip chlamydospore culture, differing markedly from one another in cultural characters, were used to inoculate a black-root-rot susceptible strain of White Burley tobacco. The roots of seedling plants were dipped in a water suspension of a 28-day-old agar culture of the organism. The plants were then set in jars of steamed soil in holes in which were placed agar cultures of the organism. An examination of some of the plants at the end of 3 weeks showed typical black root rot, but little or no difference in amount of rotting caused by the various cultures except that the albino seemed to be slightly more pathogenic than the others. After 5 weeks the amount of black root rot was the same for all cultures except the albino, which still seemed slightly more pathogenic than the others.

DISCUSSION

Gilbert (2), and McCormick (4), grew *Thielaviopsis basicola* on artificial media, but neither mentioned variability. The limited tests indicate that *T. basicola* is as unstable in culture as the smuts, Fusarium, Phytophthora, and others.

The cause of cultural variations of imperfect fungi is unknown and will be difficult of solution until more is known of their nuclear constitution. There seems to be some genetic significance to the changes observed, although they occur in what now appears to be a random manner. *Thielaviopsis basicola* may carry at least one and possibly several factors for color, factors for the production of at least 2 and possibly more kinds of spores, as well as factors that determine hyphal types. Some of these factors, such as those for the production of endoconidia and those for color appear to have been completely lost in certain cultures. The mechanism by means of which this suppression or loss of factors occurs is unnkown.

Thielaviopsis basicola, because of its unstable constitution, its many color and other physical characters, and its 2 distinct spore types, should be an ideal organism for studying the genetics of the imperfect fungi when a technic for such studies is finally worked out.

SUMMARY

Eleven cultures of *Thielaviopsis basicola* isolated from rotting roots of White Burley tobacco, differed from one another when grown on potato-dextrose agar. These cultures, after having grown a few weeks in test tubes, formed numerous sectors when transferred to potato-dextrose agar in Petri dishes, possibly due to some kind of segregation during growth in the tubes. Single-endoconidium cultures of the above differed from one another and were also unstable in culture. Single-spore cultures of single-spore cultures were as unstable as the original isolants and the single-spore cultures from which they came.

A culture producing only chlamydospores was isolated from rotting roots of tobacco. Two other chlamydospore cultures developed as sectors and none of these produced endoconidia during 1 or 2 years' propagation.

An albino culture, having both endoconidia and chlamydospores, developed as a sector in one plate. This was subcultured and kept for 4 years, but did not produce any color. Seven single-spore cultures of the albino were white and had both types of spores, although there were 3 culturally distinct types.

Eight single-spore cultures, differing in appearance, showed little difference in pathogenicity.

Sexual fruiting bodies did not develop in old cultures nor in plates containing 2 or more intermingling single-spore cultures from different sources.

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FACTORS AFFECTING EXPERIMENTAL ERROR IN GREENHOUSE POT TESTS WITH SUGAR BEETS[1]

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INTRODUCTION

Despite effort to secure relatively uniform temperature conditions within a greenhouse, it is the common experience that plants within a test series are not given the same temperature exposures. These temperature differences may be large if the heating system consists of vertical radiating coils located on the side walls or ends of the greenhouse. Obviously, the differences are still greater if some of the plants are placed close to the pipes and exposed to direct radiation.

The degree of experimental error caused by such temperature differences has been given little consideration in plant pathological experiments. The results of two experiments designed to ascertain the fluctuation in damping-off effects in a series of pots are illustrative of the magnitude of the error. The temperature records taken with calibrated thermographs at two locations on the plant tables reveal important differences that probably more or less directly influenced the incidence of damping off. Methods are suggested as to ways and means by which the variance assignable to temperature and other positional effects likely to be encountered in greenhouse tests may be determined and removed from consideration.

METHODS

The data presented were obtained in the winter of 1933 in tests to determine the capacity of soil pathogens to cause damping off of sugar-beet seedlings growing in 4-inch pots. In the first series, the plant table was a raised bed with concrete walls about 18 inches high. This bed was filled level full with soil, and the pots stood fully exposed on this solid plant table. In the second series, the pots stood on an ordinary greenhouse bench having 6-inch side walls. A vertical coil of heating pipes was situated along one side and at one end of both the concrete plant table and the board-wall bench. As shown by the photographs (Fig. 1), the raised concrete bed was more exposed to direct radiation than the board-wall bench. Calibrated thermo-

[1] The data presented in this paper were obtained in cooperative investigations by the Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Division of Plant Pathology and Botany of the Minnesota Agricultural Experiment Station. Paper No. 1322 of the Journal Series of the Minnesota Experiment Station.

[2] The writer gratefully acknowledges the helpful criticism of Drs. G. H. Coons and E. C. Stakman in the preparation of the manuscript and to Dr. F. R. Immer for aid in the statistical analyses.

graphs were maintained in the center of both sides of each plant table. As shown in figure 1, the Bourdon tube of each instrument was protected from direct radiation of the sun.

FIG. 1. Plant tables upon which uniformity studies with sugar beets were made. A. Concrete bed. B. Board-wall bench. Note the position of heating pipes and thermographs in each case.

Attempt was made to secure uniform soil conditions in the pots. Non-steamed, thoroughly mixed garden soil, into which a small amount of *Rhizoctonia solani* Kühn from sugar beet had been evenly incorporated, was used. In the first experiment, 400 four-inch pots were arranged in 40 equal rows running across the raised concrete plant table. In the second experiment, 432 four-inch pots were arranged in 36 equal rows running across the board-wall bench. Fifty seed balls of K. W. Normal sugar beets were planted in each pot, and covered to a uniform depth of one inch. Stand counts were made for each pot two weeks after planting. The data were analyzed by Fisher's analysis-of-variance method, the significance of the results being determined from the appropriate tables given by Fisher.[3]

[3] Fisher, R. A. Statistical methods for research workers. Ed. 4, rev. and enl., 307 pp., illus. Oliver and Boyd, Edinburgh and London. 1932.

RESULTS

The analyses of variance of the stand counts for each of the 400 pots on the concrete bed are given in table 1.[4] It is seen that the variance "between columns" with respect to the variance "within columns" is highly signifi-

TABLE 1.—*Analyses of variance of seedling stands in 400 4-inch pots placed on the surface of a concrete bed in the greenhouse*

Variation	Sum of squares	Degrees of freedom	Variance	z
		Between the columns		
Between columns	4,591.70	9	510.18	0.5146[a]
Within columns	71,116.05	390	182.35	
Total	75,707.75	399		
		Between the rows		
Between rows	8,281.15	39	212.34	0.0626[b]
Within rows	67,426.60	360	187.30	
Total	75,707.75	399		

[a] Exceeds 1 per cent point.

[b] Does not reach the 5 per cent point.

cant, the observed z exceeding the value of that statistic at the 1 per cent point. On the other hand, the variance "between rows" as compared with the variance "within rows" does not reach a significant value.

From the data obtained, it was seen that more seedlings emerged in the pots immediately adjacent to the heating pipes. The total for the column (1,838 seedlings) was significantly higher than the total for any other column. The seedling stands of Row 1 (next to the pipes) and Row 40 (farthest from the pipes) were higher (434 and 440 seedlings, respectively) than any of the rows immediately adjoining, but the differences between rows were not significant for the experiment as a whole; hence these variations are interpreted as due to chance.

The data for the second experiment, involving 432 pots in a board-wall bench, were similarly analyzed, and it was found from comparison of the variance "between columns" with that "within columns" that significant differences did not exist (Table 2). In the comparison of the variance "between rows" with the variance "within rows," the difference was highly significant, since the z value exceeded that for the 1 per cent point. Thus,

[4] In the calculation of variances in the experiments, the stand counts of the individual pots were (a) combined as rows running across the plant table to determine the variance "between rows" and (b) the appropriate readings for the individual pots were then considered in their lengthwise alignment on the plant table, to determine the variance "between columns." The individual readings of stand counts of the two experiments are not printed but are available to any one interested.

TABLE 2.—*Analyses of variance of seedling stands in 432 4-inch pots placed on a board-wall bench in the greenhouse*

Variation	Sum of squares	Degrees of freedom	Variance	z
		Between the columns		
Between columns	2,201.14	11	200.10	[a]
Within columns	90,021.64	420	214.34	
Total	92,222.78	431		
		Between the rows		
Between rows	17,612.78	35	503.22	0.4912[b]
Within rows	74,610.00	396	188.41	
Total	92,222.78	431		

[a] Not significant.

[b] Exceeds the 1 per cent point.

with the board-wall bench, the situation as to positional effect was the reverse of that with the concrete bed.

The temperature records as obtained from the recording thermographs, and summarized in table 3 as minimum, maximum, and mean temperatures

TABLE 3.—*Minimum, maximum, and mean temperatures on heated and non-heated sides of the concrete bed and the board-wall bench in the greenhouse*

Temperature	Type of plant table	
	Concrete bed	Board-wall bench
	°F.	°F.
Minimum		
Heated side	71.9	59.4
Non-heated side	64.2	63.8
Difference	7.7	4.4
Maximum		
Heated side	89.0	78.9
Non-heated side	81.1	82.3
Difference	7.9	3.4
Mean		
Heated side	80.4	69.1
Non-heated side	72.6	73.0
Difference	7.8	3.9

for the side of plant tables nearer the heating pipes and the side farther removed, give an indication of at least one factor that was concerned in the variation found. It is seen that, on the average, the side of the concrete bed near the heating pipes was about 8 degrees F. warmer than the side more

removed. In the case of the board-wall bench, the condition was reversed, the mean temperature for the side near the steam coil was, on the average, from 3 to 4 degrees F. lower than the side more removed.

It would seem probable that these temperature differences, in the case of either the concrete bed or board-wall bench, and especially in the case of the former, are sufficiently great to cause differences in response in tests involving soil-borne pathogens.

The analyses made indicate that there is a definite positional influence on each type of plant table. It appears clear that this heterogeneity in temperature exposure, with its attendant effects on drying of the soil, together with the recognized differences in light exposure in the greenhouse, should be considered in designing experiments.

In agronomic field work, following the suggestions of Fisher,[5] a restricted randomized arrangement of plots is utilized that permits assignment of the variance arising from place effects, and its removal from consideration in the comparison of variance to be assigned to treatment with that designated as error.

The data from these 2 uniformity tests may be used to test the value of an experimental design that would permit removal of the variance assignable to position on the plant table. An experimental design suitable for such a test would be the equalized randomized-block arrangement suggested

TABLE 4.—*Analyses of variance of the two hypothetical Latin-square arrangements of the data obtained from the uniformity tests made on a raised concrete bed and a board-wall bench in the greenhouse*

Variation due to	Sum of squares	Degrees of freedom	Variance	z
		Raised concrete bed		
Rows	1,256.20	9		
Columns	4,591.70	9		
Strains	9,536.15	39	244.52	0.1632[a]
Error	60,323.70	342	176.38	
Total	75,707.75	399		
		Board-wall bench		
Rows	12,488.64	11		
Columns	2,201.14	11		
Strains	4,053.03	35	115.80	[b]
Error	73,479.97	374	196.47	
Total	92,222.78	431		

[a] Does not exceed the 5 per cent point.

[b] Variance due to strains less than that due to error; therefore, strains are not significantly different.

[5] *Loc. cit.*

by Student.[6] Taking the data from the test on the concrete bed one may assume that 40 strains of a fungus were to be tested, using 10 replications. In this arrangement, the 40 strains as represented by 10 pots each are placed at random on the bench, the only requirement being that each strain occur but once in each row-group (4 rows) and once in each column. Such an arrangement was devised, and the values obtained in the 2 uniformity tests were taken as appropriate readings.

Table 4 gives the analysis of this hypothetical experiment on the raised concrete bed. It is seen that the variance assignable to strains is not significant. Since the disease incidence among the pots was fairly uniform, the variance originally shown (Table 1) probably was due largely to general differences in the exposures on the bed. This failure of the hypothetical strains to show significant differences is what is to be expected, provided the variance assignable to location is removed.

Similarly, if another hypothetical test of 36 strains, replicated 12 times in accord with the same experimental design, is set up using the data from the pots on the board-wall bench, positional effects may be determined and when removed strains are, likewise, found to be not significant (Table 4).

It is to be noted that in the analysis (Table 2) of the data of the second experiment, a significant difference "between rows" was found; therefore, any systematic replication of pots would have been likely to give a false indication of the situation and cause one to assign a difference, in reality due to position, to the organism that happened to be placed in that row.

SUMMARY AND CONCLUSIONS

In greenhouse tests, attempt is made to establish uniformity of conditions except for the factor under consideration. The uniformity tests reported afford indication of the variations in conditions likely to be encountered and their effects. In one uniformity test the percentage of damping off of sugar beets in the border row of pots on a raised concrete bed was markedly less than that in the adjacent rows of pots. This effect was less marked, or almost absent, in a bench with board walls, because of deflection of direct heat. Obviously, uniformity of environmental conditions would be improved by suitable devices for deflection of direct heat, and, in greenhouse experiments, the possibility of temperature differences needs to be considered. The second experiment showed that, on a board-wall bench, unsuspected environmental factors also operated that resulted in certain rows showing significantly different amounts of damping off. These differences were of such magnitude as to bias judgment had the experiment been set up in a systematic arrangement so that, for example, all inoculations with cer-

[6] Student. Yield trials. *In* Baillière's Encyclopedia of Scientific Agriculture. Vol. 2, pp. 1342–1361. Baillière, Tindall & Cox, London. 1931.

tain pathogens were located in such rows. Since these variations in environmental conditions may occur and may not be fully anticipated, the applicability of certain methods now in use in agronomic field experiments is pointed out. In meeting such problems in the field, experimental designs are used in which the variates are so randomized as to avoid localization and to permit an evaluation of place effects. It is found that the use of a suitable design not only adds to the efficiency of the experiment but also serves to take into account some, at least, of the unsuspected factors that might bias interpretation.

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THE DEMONSTRATION OF PLANT-PARASITIC NEMATODES IN HOST TISSUES

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The method of demonstratinn, by staining and clearing, of plant parasitic nematodes in the host tissues, described by Steiner (4) and later, with slight modification, by the writer (1), is here modified still further. The basic principle is the reduction of osmic acid by the fats and lipoids of the nematodes, with production of a dark color, as explained in Lee's Vade Mecum (3). While satisfactory for roots and other materials not containing excessive oils and lipoids, the old method was unsatisfactory for green plants because of the dark color produced in the tissues by quick reduction of the osmic acid of the killing fluid, which completely obscured the nematodes.

Attempts were made by various methods to eliminate from the leaves the materials that bring about this effect. Most of them failed because the reagents removed such materials simultaneously from the nematodes. A successful procedure here described finally was adopted.

Materials.—80 per cent acetone in water; Flemming's Strong killing fluid; graduated alcohols, from 15 to 100 per cent; clove oil.

Procedure.—The method, in brief, follows:

(1) Bring some 80 per cent acetone to boiling in a test tube or small Erlenmeyer flask. Since the boiling point of this solution is about 63° C., this may be done by immersing the container in hot (75 to 90° C.) water, or even by allowing some hot tap water to flow over it.

(2) Quickly submerge in the hot acetone solution small pieces of leaves, stems, or galls known or suspected to contain nematodes. The nematodes will be killed promptly without appreciably modifying their internal structure, and the lipoid-extracting process will then commence. Leave the materials in the gradually cooling solution for 3 to 4 hours or until the chlorophyll is removed.

(3) Pour off the acetone and wash the materials in 3 or 4 changes of water.

(4) Immerse the materials in Flemming's Strong killing fluid. This may be done in a Syracuse dish under the binocular in order to observe the depth of coloration of the nematodes and the contrasts obtained.

(5) Wash to remove the killing fluid; dehydrate in alcohol; and infiltrate and clear with clove oil. Clove-oil mounts of the materials may now be made for temporary observation or for photographing.

(6) For permanent mounts, replace the clove oil with Canada balsam.

Practice will indicate the desirable periods of exposure for each of the reagents used. The boiling point of pure acetone is 56.4° C. By diluting this slightly in water, not only is the lipoid-extracting process retarded and less violent, but the boiling point is slightly raised—both, desirable features. The boiling point of 80 per cent acetone, about 63° C., is not

FIG. 1. A garlic plant infested with the stem and bulb nematode, *Anguillulina dipsaci*. The nematodes are abundant in the outer bulb scales and outer leaves, though they are not evident on superficial examination.

too high for satisfactory nematode fixation. The danger of excessive heating is thus eliminated. The extraction, of course, must not be carried too far; otherwise the nematodes will not become colored. The desired point is best reached for demonstration of the nematodes in the tissues by occasionally removing a piece of the material and testing it with Flemming's fluid. As soon as the plant tissues no longer quickly turn dark, the rest

of the materials may safely be removed and subjected to the Flemming's treatment. Usually from 5 to 10 minutes in this solution suffices for demonstrating the nematodes. If they do not show up in this time the extracting process probably has been carried too far.

FIG. 2. A small block clipped from the side of a green leaf of a nematode-infested garlic plant, prepared according to the method herein described. Nematodes in all stages of development are clearly differentiated in the leaf tissues. ×16.

Figure 1 is a garlic plant heavily infested in the bulb scales and outer leaves by the stem and bulb nematode, *Anguillulina dipsaci*. In green parts of the leaves the swelling and distortion commonly produced in plants by this nematode were lacking, so nematode invasion was not demonstrable except by dissection. Rectangular blocks of full thickness

were taken from infested bulb scales and green leaves and processed according to the foregoing method, both with and without the acetone treatment. Those without the acetone became quite black and the nematodes were obscured, except for an occasional individual protruding from the cut edge. Figure 2 is a photomicrograph of a green leaf block receiving the full process, as it appears mounted in clove oil in a hollow slide. Eggs, larvae, and adults are very sharply defined, just as they lie in the leaf tissues. A block from the side of a parsley leaflet containing the same nematode is pictured by Godfrey and Scott (2, fig. 2, A). The writer has held such materials for nearly two years without reduced contrast.

With acetone as the killing medium, the writer has been able to control the period of extraction to the point that some of the fats were removed from the nematodes, so that subsequent exposure to Flemming's fluid darkened them only slightly. In this way he has been able to bring out very clearly the internal organs of the nematodes including the very sharply delimited ovaries and testes. Whether or not this method can be adapted to differential staining technique with the usual nematode stains, remains to be determined by further investigation. The block of plant tissue constitutes a convenient medium in which the nematodes can be carried through the various differentiating processes assembled in one piece of convenient size for handling. At any desired stage they can be removed by dissection and given further individual processing.

SUMMARY

The staining of plant-parasitic nematodes within the host tissues that results from treatment with Flemming's killing fluid is due to the reduction of the osmic acid by the fats and lipoids of the nematodes. Frequently the plant tissues become black as well, due to the contained reducing agents of one kind or another, and the nematodes are obscured. This is particularly true with green leaves and stems. This difficulty can be overcome by first treating the blocks of plant materials momentarily with hot, slightly dilute acetone, which kills the nematodes quickly in their natural position in the host tissues; then subjecting them to the extractive influence of the solution for 3 or 4 hours. This removes the chlorophyll and at the same time removes, at least in part, the lipoids and other reducing substances. The specimens can then receive the regular Flemming's treatment, followed by washing, dehydration in alcohols, and clearing, with excellent results in nematode differentiation.

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DIFFUSIBLE NATURE OF THE INHIBITORY AGENT PRODUCED BY FUNGI[1]

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INTRODUCTION

The growth of specific fungi on certain substrates causes the latter to be altered. Further growth of a fungus on such an altered substrate may be retarded or entirely prevented. A substrate thus affected is said to have become stale. Each fungus produces a characteristic type of growth on a given substrate. When one fungus is grown in association with another, the characteristic type of growth of each may be altered. On a solid substrate the organisms may grow and meet. One mycelium may overgrow the other or it may intertwine with the hyphae of the associating organism. In other associations the organisms may never make contact because of the production of inhibitory substances. Here again the substrate becomes stale, and the organisms are prevented from making contact with each other. This phenomenon of inhibition occurs when 2 or more organisms that grow in association with each other hinder or prevent the expected development of any one or all of the organisms concerned.

Such retardation or prevention of fungus growth and spore germination is discussed by Bisby (1), Brown (4), and Pratt (17). The work of Boyle (2), Brown (5), Fahmy (10), Frost (11), Machacek (14), Porter (15, 16), and Pratt (18) demonstrated some of the characteristics of a medium that had been staled by fungus growth. Broadfoot (3) and Henry (12, 13) studied the phenomenon of inhibition of microorganisms when they grew in association with each other under natural conditions. The work of Endo (7, 8, 9) demonstrated that the reactions of microorganisms that grow in association with each other are influenced by staling products in many associations. The inhibitory effect on the growth of one organism by the products of growth of another associating organism was utilized by Coons and Strong (6) to differentiate species of Fusarium. This effect was used by Stevens and Ragle (19) to differentiate races of Sclerotium.

DISCUSSION

The observations and results of many investigators as previously indicated show that many different microorganisms exhibit the phenomenon of inhibition when in association with other microorganisms. In the present studies several of the more common parasitic and saprophytic microor-

[1] Submitted in partial fulfillment for the Ph.D. degree.

[2] The writer gratefully acknowledges the counsel given by Dr. C. L. Porter.

ganisms were grown in association with one another in order to determine the relative degrees of inhibition that would be demonstrated by different combinations of microorganisms. *Helminthosporium sativum* was inhibited consistently by a bacterium that was listed as 9a2. These two organisms, when grown in association on potato-dextrose agar, inhibited the growth of one another at distances of 10 to 15 mm.

The following procedure was used to obtain inhibition of growth of these organisms. Sterile Petri dishes were supplied with approximately 20 cc. of sterile potato dextrose agar. *Helminthosporium sativum* was planted in the center of the solidified agar. After the fungus had grown for 24 to 48 hours the bacterium 9a2 was planted in a complete circle around the fungus and as near the border of the Petri dish as was convenient. Within four to six days inhibition of the growth of both organisms was evident. This was shown by a 10 to 15 mm. sterile region between the two organisms. This sterile agar is considered to be stale because it has the ability to inhibit the further growth of *H. sativum*.

A portion of the stale agar was collected, placed in test tubes, and autoclaved at 15 pounds' pressure for 20 minutes. It was then poured into sterile Petri dishes and allowed to solidify; then, it was cut into rectangular sections. Each section was placed on agar a few mm. from the advancing circumference of *Helminthosporium sativum*. In like manner fresh agar sections, and stale agar sections that were not autoclaved, were placed before the growing circumference of *H. sativum*. These constituted checks. Growth of *H. sativum* was inhibited in a region about the sterilized and non-sterilized sections, but was not retarded by the fresh agar sections. The stale agar after it was subjected to autoclaving at 15 pounds for 20 minutes was as active in preventing the further growth of *H. sativum* as was the stale agar that was not autoclaved. It is evident that the inhibitory agent in the stale agar not only prevented the fungus from growing over the stale agar, but diffused out of the stale agar sections into the fresh agar; thus, preventing the fungus from growing to the stale agar sections.

After finding that the inhibitory agent diffused from the stale into fresh agar, an attempt was made to cause the inhibitory agent to diffuse from the stale agar into water.

The agar staled by *Helminthosporium sativum* and bacterium 9a2 was placed in 100 cc. quantities of sterile distilled water in 250-cc. flasks. All aseptic precautions were followed in transferring the agar. The stale agar was removed from the water after 48 hours. This water containing the diffusible ingredients of the agar may be designated as "stale water." The stale water was substituted for distilled water in preparing potato-dextrose agar, which may be designated as stale-water agar. The type and rate of growth of *H. sativum* on this medium was compared with that on potato-dextrose agar after 7 days' growth.

The average diameter in many tests of colonies of *Helminthosporium sativum* on normal potato-dextrose agar in 7 days was 80 mm., while that on stale-water agar was only 20 mm., or an average difference of 60 mm. The maximum difference in growth on the 2 agars at the end of 7 days was 81 mm. There was excessive spore production on the stale-water agar.

The agar that contained the diffusible products from 9a2 and from *Helminthosporium sativum* was tested for potency of the inhibitory agent. The agar from the 9a2 caused a greater inhibition of the growth of *H. sativum* than that from the *H. sativum*. However, the agar from the associated growth of 9a2 and *H. sativum* caused a greater inhibition of the further growth of *H. sativum* than the agar from the 9a2 alone. Thus, the 9a2 is more important in the production of the inhibitory products than *H. sativum*.

Helminthosporium inaequalis, when grown on stale-water agar, was inhibited like *H. sativum*. However, *Fusarium conglutinans* was not inhibited noticeably when grown on stale-water agar.

CONCLUSIONS

1. Certain combinations of microorganisms exhibit a specific antagonistic action toward one another.

2. Stale agar, produced by the growth association of *Helminthosporium sativum* and bacterium 9a2, will inhibit the further growth of *H. sativum*.

3. *H. sativum*, growing on potato-dextrose agar, will not grow in contact with stale agar that has been placed several millimeters beyond the growing fungus; thus showing that the inhibitory agent in the stale agar diffuses into the fresh agar.

4. The inhibitory agent present in the stale agar is thermostable.

5. The inhibitory agent will diffuse from stale agar into water and will retain its inhibitory properties after sterilization.

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CULTURAL STUDIES ON THE VIRUS OF TOBACCO MOSAIC

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Of the many studies that have been made on tobacco mosaic, relatively few have dealt with attempts to culture the virus *in vitro*. These studies are characterized either by negative or equivocal results (2, 3, 5, 6, 7, 8). In the present state of our knowledge, however, it is not possible to say definitely whether the virus is or is not of the nature of a living principle, or whether it can or cannot be cultured *in vitro*. The purpose of this note is to communicate some results of attempts made at Duke University, 1931 and 1932, to culture the virus.

The healthy tobacco plant was regarded as the most likely source of suitable constituents for a culture medium; and wherever possible sterilization of the medium by autoclaving was avoided in order not to add to the changes of the natural constituents of the plant during the preparation of the medium. In all cases the absence of the virus in noninoculated media was made certain by inoculating plants. A given amount of medium (usually 9.9 cc. in a test tube) was inoculated with a given amount of virus (usually 0.1 cc. of sap from mosaic plants, rendered bacteria-free by passing through a Berkefeld "W" filter) and incubated 7 days at 30° C. The infectivity of the culture was then tested by inoculating tobacco plants, and at the same time a sub-plant was made into another tube of the same medium. Successive sub-plants were made in this manner, testing the infectivity at each stage through dilutions of 1:1,000,000. The original inoculum was never found to be infectious in a dilution of more than 1:100, and sometimes not even that, probably as a result of the preliminary filtration (4). Both aerobic and anaerobic cultures were maintained, the latter by means of a layer of toluol, which, itself, evidently exerts no harmful action on the virus (1).

A summary account of the culture media prepared is a follows:

First series, from the sap of healthy plants. Fresh leaves were ground, the sap expressed under 10,000 pounds' pressure, and centrifuged. The supernatant liquid was extracted successively with benzol, ether, chloroform, and petroleum ether, retaining after each extraction a portion to be used as culture medium following sterilization by filtration. Cultures inoculated with portions extracted with benzol alone gave no indication of an increase in potency for infection, but in those extracted with both benzol and ether, as well as those extracted further with chloroform and petroleum ether, infections resulted from the 1:1,000,000 dilution, although the inoculum for the cultures was not infectious when diluted 1:100. When

the whole experiment was repeated, the results were not duplicated, for there was no such indication of an increase in potency for infection.

A solution of some of the proteins contained in the sap was prepared by completely saturating the sap with ammonium sulphate at room temperature, and filtering and resuspending the precipitate in a small amount of distilled water. This was dialyzed through a collodion membrane in running water, diluted with a phthalate buffer at a pH of 5.4 to 5.6, and filtered through a Berkefeld "W" filter. Using the filtrate as a culture medium, there was no evidence of an increase in infective power.

Second series, from fresh, unground, healthy leaves. The leaves were extracted thoroughly with 95 per cent alcohol, washed with anhydrous ether, ground to a powder in a mortar, and desiccated over calcium chloride. For a culture medium, 2 per cent by weight was added to a phthalate buffer solution at the above pH, allowed to stand for a while at 30° C., and filtered through a Berkefeld "W" filter. There was no increase in infective power of the virus when implanted in this medium.

In a third series of experiments, fresh, healthy leaves were surface-sterilized by Hexylresorcinol solution (S.T. 37) and by a mercuric chloride solution, washed in sterile distilled water and placed in a sterile cloth that was then lowered into a container of liquid air for an interval of 15 minutes. The frozen leaves were ground, as asceptically as possible in a sterile mortar and transferred to flasks containing sterile distilled water. These were used as culture flasks, with negative results.

The benzol, ether, and cholorform extractives of the healthy plant sap, as well as the alcohol extractives of the leaves, after removal of the solvents and autoclaving in phthalate buffer solution, exerted no apparent action on the potency for infection of the virus contained in the filtered sap from mosaic plants.

It would hardly be worth while to list the minor variations of the general procedures mentioned above. A total of over a thousand plants were inoculated in testing the infective power of the various "cultures," without getting any evidence of an increase in infective power except in the one instance cited. As Olitsky has pointed out (6), the apparent increases in potency for infection that are found in cultivation experiments are difficult to interpret. The increase that appeared in the present study was not duplicated in a repetition of the experiment.

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PHYTOPATHOLOGICAL NOTES

An Effective and Easily Applied Method of Inoculating Seed Barley with Covered Smut.—Progress in a study of the physiologic forms of *Ustilago hordei* (Pers.) K. and S. and in the selection and breeding of resistant barleys has been seriously retarded because no effective and easily applied method of seed inoculation has been known. Investigators repeatedly have failed to obtain high percentages of covered smut (*U. hordei*) in plants from seed blackened with spores. Previously it has been necessary to remove the seed hulls before inoculation in order to obtain satisfactory smutting.

Results of the past 2 years have shown the frequent presence of *Ustilago hordei* mycelium in or on the pericarps of seed from smutted crops that had been thrashed and stored under usual farm conditions. This may explain the failures to get smut by dusting spores on the seed surface followed by sowing the seed soon thereafter, and the failures to obtain control of covered smut in farm-stored barley through seed dusting with copper carbonate. Studies on seed inoculation, therefore, were conducted to place inoculum under the hulls of barley kernels. Numerous attempts resulted in the discovery of a relatively easily applied and highly effective method of covered-smut production. This method, as used in a study of physiologic forms of *U. hordei*, is as follows: Six-gram lots of seed are placed in $\frac{7}{8}$ in. x 3 in. shell vials. A spore suspension, consisting of 1 gram of spores added to a liter of water and thoroughly shaken, is poured into the vials until the fluid rises about $\frac{3}{4}$ in. above the seed. The seed is vigorously shaken in this suspension for $\frac{1}{2}$ minute then allowed to soak 15 minutes. The suspension then is decanted and the vials are inverted on clean pieces of blotting paper to absorb all free water. Next, the vials of moistened inoculated seed are packed in a tightly covered tin box ($5\frac{1}{2}$ in. high, 12 in. wide and 17 in. long) floored with a moistened blotter and incubated for 24 hours at 20° C. Lastly the seed is transferred to small envelopes, crimped to remain wide open, where it is left 2 or 3 days or until thoroughly dry. It is then ready for sowing. Through the cooperation of the New York (Cornell) Agricultural Experiment Station, field plantings of spring barley inoculated by this spore-suspension method were made at Ithaca, New York, in 1934 and again in 1935, and up to 70 per cent of smutted heads were obtained. Similar results have been obtained at Statesville, North Carolina, and at Rosslyn, Virginia, with winter barleys. The results at Statesville were obtained through the cooperation of the North Carolina Agricultural Experiment Station and the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. The method approaches a combination of the simplicity of dusting seed with spores and

the effectiveness of inoculating dehulled seed with dry spores. Because it is a wet method, spores do not fly about, and, therefore, it is ideally adapted for use in studies of physiologic forms. In this method, much less inoculum is needed than in the dusting method of inoculation, and, as a result, there is a consequent economy in time and greenhouse space necessary to increase small collections. To date, the method has not been injurious to seed germination. More recent studies have shown that the effectiveness of the new method may be further increased by first slightly sprouting the seed, then thoroughly drying it before proceeding with the method of inoculation described above. It is important to note, however, that the seed should be sprouted only to the extent that the radicle barely breaks through the seed-coat.—V. F. TAPKE, Bureau of Plant Industry, Washington, D. C.

The Transmission of Ceratostomella ulmi through Root Grafts.—During investigation and control work on the Dutch elm disease, cases have been observed in which transmission had undoubtedly been accomplished through root grafts between a tree supposedly inoculated in the usual manner and a nearby tree.

Root grafts between elm trees are common, especially in crowded stands. Whenever two roots cross, growth pressure becomes sufficient, as the roots increase in diameter, to flatten them at the point of contact until a complete union results. Excavations of roots of nearly a hundred pairs of elm trees show that nearly all trees over 8 inches in diameter are connected by root grafts if the stems are 7 feet or less apart. The larger the trees the greater the distance at which they will graft. Grafts have been found between trees 17 feet apart, and groups of 3 to 5 trees have been found grafted into a continuous system.

A complete vascular union in root grafts is evidenced by 3 kinds of observations: (1) Roots will live at least 3 years from a graft outwards after the death of that portion between the graft and the parent; (2) dyes can be passed readily from a root of one tree into roots of another through a graft; and (3) the vessels discolored by *Ceratostomella ulmi* (Schwarz) Buisman can be traced through grafts (Fig. 1).

During the spring and summer of 1935 12 trees showing the Dutch elm disease were found grafted to the roots of the stumps of trees known to have been diseased and removed in the regular control program of 1934. The discoloration, from which *Ceratostomella ulmi* was isolated, was traced in each case from the stump, through the grafted roots, and into the stem of the adjacent tree. In 5 of these cases the discoloration in the tree was limited to the grafted roots, collar, and lower stem but absent from the crown. Thus the fungus quite evidently had not entered through the crown, as it does ordinarily, but had entered through the grafted roots. The

FIG. 1. Roots of two elm trees grafted together. Bark removed to show streaks caused by *Ceratostomella ulmi* passing from one root into the other through the graft.

discoloration was too general in the other 7 trees to be sure that they were not inoculated in the crowns also. However, regardless of whether they were inoculated also in the crowns, the fungus had passed through the grafts.

Streaks from which *Cerastostomella ulmi* was isolated, were traced through root grafts between 5 pairs of stumps removed in the 1934 control program. Nothing is known of the discoloration in these trees at the time of felling to indicate the points of inoculation, but it is possible that *C. ulmi* could have been transmitted to one tree of each pair through the grafted roots and that each would become diseased, even had there been no crown inoculations.—A. F. VERRALL AND T. W. GRAHAM.[1]

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